



# CEREAL CHEMISTRY

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## CONTENTS

	PAGE
Studies on the Importance of Starch and Protein Systems of Individual Flours in Loaf Volume Production. <i>J. W. Pence, Kathryn M. Eremia, N. E. Weinstein, and D. K. Mecham</i> . . . . .	199
Hydroxyethylated Cereal Flours. <i>J. C. Rankin, C. L. Mehltretter, and F. R. Senti</i> . . . . .	215
Some Factors That Affect the Staling of White and Yellow Layer Cakes. <i>K. Kulp, J. G. Ponte, Jr., and W. G. Bechtel</i> . . . . .	228
Relation of the Rigidity of Flour, Starch, and Gluten Gels to Bread Staling. <i>J. E. Cluskey, N. W. Taylor, and F. R. Senti</i> . . . . .	236
A Study of the Effect of Drying Conditions on the Suitability for Starch Production of Corn Artificially Dried After Shelling. <i>Majel M. MacMasters, M. D. Finkner, Margaret M. Holzapfel, J. H. Ramser, and G. H. Dungan</i> . . . . .	247
Water Penetration and Internal Cracking in Tempered Wheat Grains. <i>Gordon M. Grosh and Max Milner</i> . . . . .	260
Gas Exchange Induced in Dry Wheat Embryos by Wetting. <i>Pekka Linko and Max Milner</i> . . . . .	274
Free Amino and Keto Acids of Wheat Grains and Embryos in Relation to Water Content and Germination. <i>Pekka Linko and Max Milner</i> . . . . .	280
Studies with Radioactive Tracers. II. The Presence of Unchanged Bromate in Bread. <i>C. C. Lee and R. Tkachuk</i> . . . . .	294
Observations on Food Preferences of Five Species of Stored-Product Insects. <i>S. R. Loschiavo</i> . . . . .	299
Relationships between Fat Acidity Values and Types of Damage in Grain. <i>Doris Baker, M. H. Neustadt, and Laurence Zeleny</i> . . . . .	307
Quantitative Relation between Structural Relaxation and Bromate in Dough. I. <i>Hlynka and R. R. Matsuo</i> . . . . .	312
Editorial Policy . . . . .	318

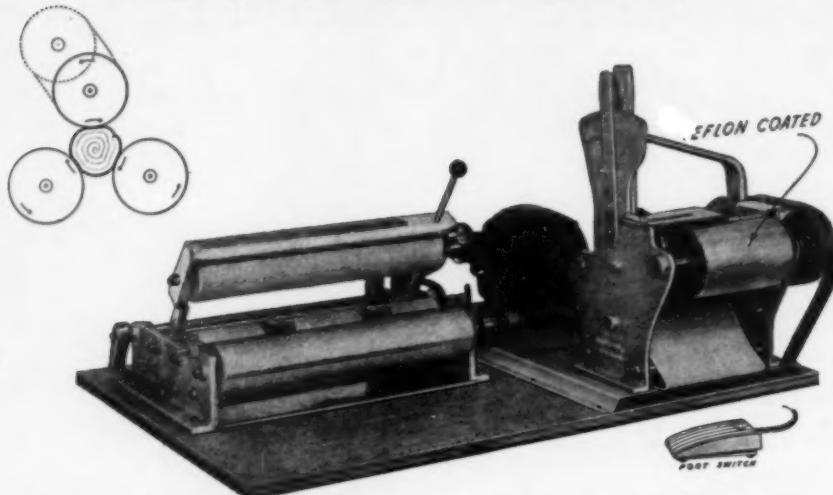
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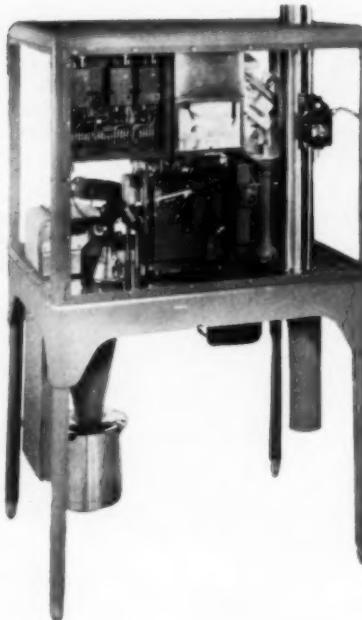
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# CEREAL CHEMISTRY

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## STUDIES ON THE IMPORTANCE OF STARCH AND PROTEIN SYSTEMS OF INDIVIDUAL FLOURS IN LOAF VOLUME PRODUCTION<sup>1</sup>

J. W. PENCE, KATHRYN M. EREMIA, N. E. WEINSTEIN,  
AND D. K. MECHAM

### ABSTRACT

Relationships between protein content and loaf volume were determined for a number of flours of widely varying type and quality by (a) dilution of each flour with its own starch or enrichment with its own protein components; (b) enrichment of a low-protein base flour with protein components of the different flours; and (c) interchange of starch and protein components of three of the flours in completely reconstituted doughs.

Regression lines for the various flours could be grouped into three rough types on the basis of their vertical placement and on the manner of their approach to a common intercept at very low protein levels. Enrichment of a low-protein flour with proteins from other flours decreased differences in vertical location, apparently because of differences in starches among the original flours. However, the method appears less satisfactory for estimation of protein quality than the enrichment and dilution of flours with their own components. Results with the reconstituted doughs indicated that protein, starch, and protein-starch interaction effects are all significant factors governing flour quality. Protein effects appear to govern slope values of regression lines primarily, whereas starch effects appear to influence vertical placement of the lines. Starch also appears to be able to influence slope values in some cases.

In the light of present knowledge, protein adjustment of a flour with its own components to obtain the coefficient of regression of loaf volume on flour protein appears to provide a sound method by which to estimate for research purposes the baking quality of the proteins of flour. By this single parameter, the protein of flours of different type and variety can be compared, despite differences in protein content. Much additional work will be required, however, to make the method completely satisfactory.

In studies concerned with the chemical composition of wheat flours and their quality performance, objective measurements of quality factors that can be expressed by a single parameter are highly desirable. Of the several factors of importance for bread flours, loaf volume-producing capacity is one of the most important, although in commercial usage much emphasis is currently placed on mixing time and tolerance,

<sup>1</sup> Manuscript received May 5, 1958. Contribution from Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture. Presented at the annual meeting, April 7-11, 1958, Cincinnati, Ohio.

absorption, etc. A number of investigators (1,3,6,8,12, among others) have established that loaf volume varies linearly with flour protein over a wide range, provided that baking tests and formulas are suitable, and that within a class of wheat the slope of the regression of loaf volume on flour protein is a valid varietal characteristic. For example, Finney and Barmore (6) found that regression lines for hard red winter wheat varieties tended to converge at about 7 to 8% protein and to fan out at higher protein contents, with few lines crossing above this point. Hard red spring wheats showed a less marked convergence to a common point, with crossing of many of the regression lines above 7 or 8% protein (6). These authors proposed a method for evaluation of new wheat varieties by use of an idealized array of lines and correction of loaf volume values to a common protein content.

Straightforward application of this method is not possible, however, if varietal regression lines cross each other or lie at different levels on the frame of reference within the common range of protein percentages. McCalla (8) found both types of deviation to interfere in a direct comparison of the loaf volume-producing capacity of flours. When regression lines cross one another within the range of usual protein contents, an uncertainty is introduced as to what protein level should be used for comparison of loaf volumes. The wider the range of protein contents of flours to be compared, the more serious the uncertainty becomes. When regression lines of similar slope are situated well above or below one another, a second parameter (intercept of the regression line) is necessary to describe the comparison completely, unless the lines converge toward a common point as found by Finney and Barmore (6) with hard red wheats.

In a previous paper from this laboratory (10), regression values were presented for a number of individual flours which had been diluted or enriched with their own starch or protein components so as to provide several protein levels for each flour. As only the protein contents of each flour had been manipulated to an appreciable extent, the slopes (regression coefficients) of the lines were assumed to represent estimates of the loaf volume-producing qualities of the *protein systems* of the individual flours. No inference seemed justified that such lines should necessarily agree with similar lines determined for a series of flours of different protein contents within a variety.

A considerable degree of crossing and vertical dispersion of the regression lines within the range of usual protein contents was obtained for the group, as the flours represented a wide variation in type, natural protein content, and bread-baking quality. In agreement with the experience of McCalla (8), the lines could be classified into two or

more roughly defined groups. Many of the flours, usually from soft wheats, gave rather good loaf volumes at relatively low protein levels, but failed to show a good increase in loaf volume at higher protein contents—that is, their slope values were small. Most hard wheat flours from varieties generally considered good gave very poor loaf volumes at low protein levels, but increased rapidly in loaf volume as their protein content was raised. A few others from poor varieties not only gave poor loaf volumes at relatively high protein levels but also failed to show a very good increase in loaf volume as their protein content was raised further. The latter group includes varieties such as Chiefkan, Red Chief, etc. Extrapolation of regression lines of the first type to very low protein values would require a sigmoid type of flexure for convergence to a common loaf volume at zero protein content, rather than the hyperbolic shape found by Finney (4) for three protein-adjusted hard wheat flours, including two hard winter and one hard spring wheat.

The present paper describes results of a more detailed examination of relationships between loaf volume and protein content of individual flours, carried out in an effort to assess the validity of regression lines as a quality measure of the *protein* systems of flours of *all* types. Baking experiments were made with flours adjusted to various protein contents with their own components, with a low-protein base flour to which protein components of other flours were added, and with totally reconstituted doughs in which protein and starch systems of different flours were interchanged. The results show that starch systems of flours can be very important with respect to vertical placement and sometimes the slope of regression lines. Nevertheless, the slope of the regression lines still seems to be the best measure of protein quality available.

### Materials and Methods

The unbleached, straight-grade flours used were milled from stocks of pure varieties of wheats selected to represent widely differing types, as shown in Table I. Most of the flours were milled from wheats harvested from 1954 to 1956, with the exception of Yorkwin wheat which was harvested in 1946. In this laboratory flours are routinely stored in sealed cans at  $-10^{\circ}$  to  $-20^{\circ}\text{F}$ . ( $-23^{\circ}$  to  $-29^{\circ}\text{C}$ ). Critical baking tests have shown a relatively constant baking performance for such frozen flours for at least 11 years. The experimental work presented here was done during late 1956 and early 1957.

Flours were separated into gluten, total solubles, and starch by washing doughs with an approximately constant ratio of 0.1%, pH 6.8 phosphate buffer (4 to 5 liters per kg. of flour). Glutens were frozen

TABLE I  
DESCRIPTION OF FLOUR SAMPLES

VARIETY	TYPE	PROTEIN CONTENT <sup>a</sup>	ASH CON- TENT <sup>a</sup>	TYPE OF MILLING
Yorkwin	Soft white	7.6	0.39	Commercial
Unknown	Durum (California)	9.5	.64	Commercial
Seneca	Soft red winter	9.6	.50	Commercial
Unknown	Durum (No. Dakota)	11.0	.80	Experimental
Baart	White spring (1955)	11.3	.53	Experimental
Thatcher	Hard red spring (1955)	11.4	.29	Commercial
Red Chief	Hard red winter (1955)	12.2	.47	Commercial
Idaed	White spring	12.4	.46	Experimental
Mida	Hard red spring	12.6	.35	Commercial
Thatcher	Hard red spring (1954)	13.3	.31	Commercial
Kiowa	Hard red winter	14.2	0.34	Commercial

<sup>a</sup> At 14% moisture.

in thin sheets on dry ice, broken into small pieces, and stored in the frozen state in sealed containers. Starch was centrifuged from wash waters, dried at about 45°C., ground with a high-speed hammer mill, and stored in sealed glass jars. Wash waters from which the starch had been centrifuged were concentrated under vacuum below 30°C. to a thick syrup which was then dried by lyophilization. Glutens and total solubles were added back to flours or reconstituted doughs on the basis of their protein contents ( $N \times 5.7$ ) and in the same proportions in which they were recovered from the original flours.

Moisture contents of flours, glutens, and starches were determined by drying 18 to 24 hours in a vacuum oven at 55° to 60°C. Moisture contents of solubles fractions were not determined, although precautions were taken to avoid undue moisture increases because of their slightly hygroscopic nature. Protein ( $N \times 5.7$ ) contents of glutens ranged from about 75 to 82%; of starches from about 0.5 to 0.8%; and of solubles fractions from about 13 to 23%. Percentages of flour nitrogen that were obtained in the solubles fractions ranged from about 6.5 to 12.5%, depending chiefly on the flour used. For example, values for Thatcher and Red Chief ranged from 6.2 to 7.6%; for Seneca from 8.8 to 9.8%; and for durum flours from 11.5 to 12.5%.

The formula and baking procedure recommended by Finney and Barmore (5) were used, except that 0.25% of malted wheat flour was substituted for malt syrup. Baking pans were the AACC standard low-form pans. Optimum mixing times, absorptions, and levels of bromate were determined for each flour or reconstituted dough by extensive preliminary baking tests. This was particularly important for the totally reconstituted doughs, because they were quite sensitive to minor variations in these factors.

Regression equations were calculated from loaf volumes at four or more levels of protein. Baking trials at each protein level were replicated at least once and on different days.

### Experimental Results

*Dilution of Flours to Low Protein Levels.* Baking results for a number of flours enriched with their own protein components (glutens and solubles) or diluted with their own starches are summarized graphically in Fig. 1, in which the curves were fitted by inspection. Examples

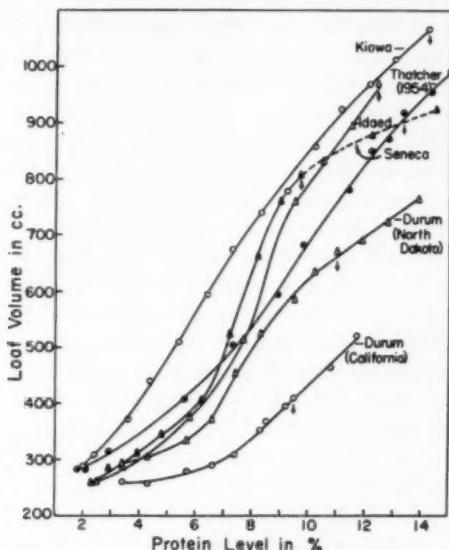


Fig. 1. Loaf volumes of flours adjusted in protein content by dilution with their own starch or enrichment with their own gluten and total solubles fractions. The small vertical arrows indicate values for each flour at its natural protein content. The dashed portion of the line for Seneca represents some uncertainty that these values were obtained under optimal baking conditions.

of typical behavior are included, along with the extremes of behavior that have so far been encountered. Dilutions to very low protein values were included to illustrate the different types of behavior that have been observed in this abnormal region.

Ordinarily, linear regression lines with flours and their components are obtained easily enough, so long as protein contents of the flours are adjusted within a range of a percent or two of their original protein content. Occasional difficulty in achieving a linear behavior at protein

levels more than 2 or 3% above the original protein content of the flours can perhaps be attributed to inadequate or improper experimental techniques. Other workers have obtained linear behavior with protein-enriched flours up to 20% protein, or higher (1,4).

The curve shown for Thatcher (1954) flour is typical of most flours from hard red spring or winter wheats. The line maintains a rather steep linear course until protein levels fall to about 7 to 8%, whereupon it begins to curve toward an intercept common with other flours at zero protein. Finney (4) found such behavior with all three of the protein-adjusted flours he used, and such behavior is implied for regression lines obtained for many other hard red varieties (3,6,8). The Kiowa flour used in the present study exhibits the most extreme behavior that we have yet encountered among strong flours. Its curve retains an almost linear slope to below 3% protein.

The curves in Fig. 1 for Seneca and Idaed flours are typical of many of the soft wheat flours studied in this fashion. (The dashed upper portion of the line for Seneca flour indicates an uncertainty that optimal mixing, absorption, or oxidizing treatments were used for these points. Unfortunately, the baking experiments had to be terminated before this possibility could be checked.) Many of these flours produce rather good loaf volumes at their original protein content. In fact, at 8 to 10% protein, they are usually superior in loaf volume to hard wheat flours of similar protein content (see also 3, 6, 8). However, many soft wheat flours show a pronounced sensitivity to dilution with starch, so that a sigmoid type of flexure of their regression lines is obtained as they approach zero protein content. In most cases this sharp downward inflection of the line seems to appear in the region between 7 and 9% protein. Some soft wheat flours, however, are tolerant to starch dilution to 7% protein, or lower. Many soft wheat flours produce excellent loaf volumes and linear regressions of constant slope at protein contents rising well above 10%. The Idaed flour is a good example, although similarly good performance has been obtained with other flours having much lower natural protein contents and whose regression lines showed good linearity above and below their natural protein contents (10). The small slope of the upper portion of the Seneca line, however, exemplifies the poor baking performance of many soft wheat varieties of protein-enriched soft wheat flours at higher protein levels.

The regression line for California durum exemplifies, in the extreme, another type of baking behavior found mostly with poor-quality hard wheats such as Red Chief or Chiefkan and durums. Loaf volumes even at relatively high protein levels are very small. With many

such flours, however, rather respectable increases in loaf volume result from enrichment with extra protein. Regression coefficients (slopes) often are larger than those of some soft wheat flours and occasionally are as large as given by some flours from hard wheat varieties normally considered of high quality. These observations agree reasonably well with those of Finney and Barmore (6) and of Harris (7) for flours and glutens from flours of the Chiefkan and durum types.

*Addition of Flour Proteins to a Low-Protein Base Flour.* The starch in flours has long been recognized as an important factor affecting physical properties of doughs and baking performance of flours (11,13), although its importance is usually considered secondary to that of the

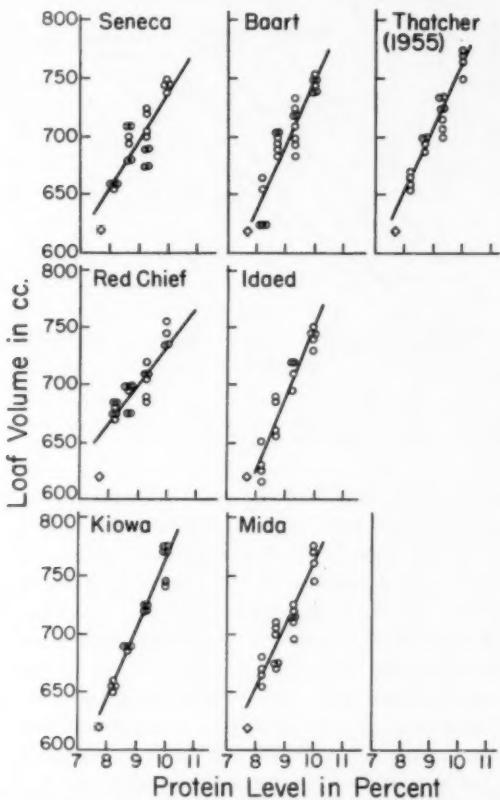


Fig. 2. Loaf volume values obtained by adding gluten and water-solubles from individual flours to a low-protein base flour (Yorkwin variety). Diamond-shaped symbol indicates protein content and average loaf volume for the base flour.

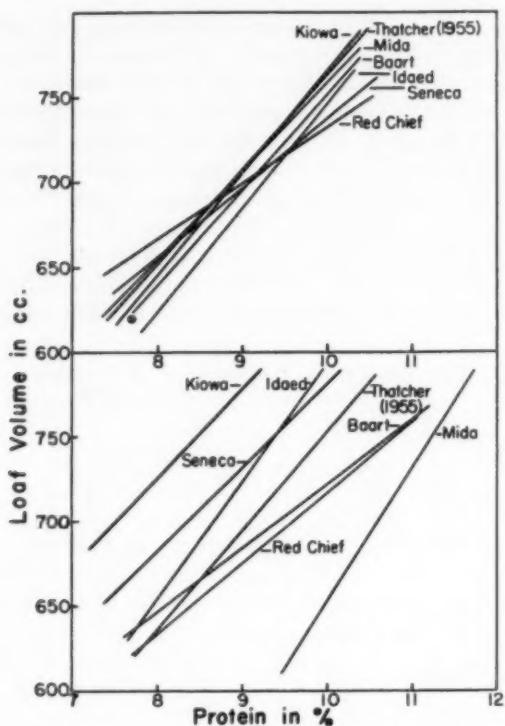


Fig. 3. Regression lines obtained by enrichment and dilution of flours with their own components (lower part of figure) and by enrichment of a low-protein base flour (Yorkwin variety) with proteins from the same flours (upper part of figure).

proteins (3,6). Possible causes of the wide differences in vertical placement of regression lines such as those shown in Fig. 1 could be differences in the starch systems of flours or interaction effects between starches and proteins. A method that has been used to eliminate differences due to starches when comparisons of baking quality were desired is addition of the test materials to a low-protein base flour (7,12). This method suffers from the disadvantage that a blend of proteins is actually being tested. Harris (7), for example, found a remarkably small differentiation among glutens from flours of widely differing quality. This, however, could have resulted from failure to include water-soluble components from the original flours along with glutens to the base flour. Earlier experiments from this laboratory and elsewhere (4,9) showed that differentiation between glutens in baking tests with

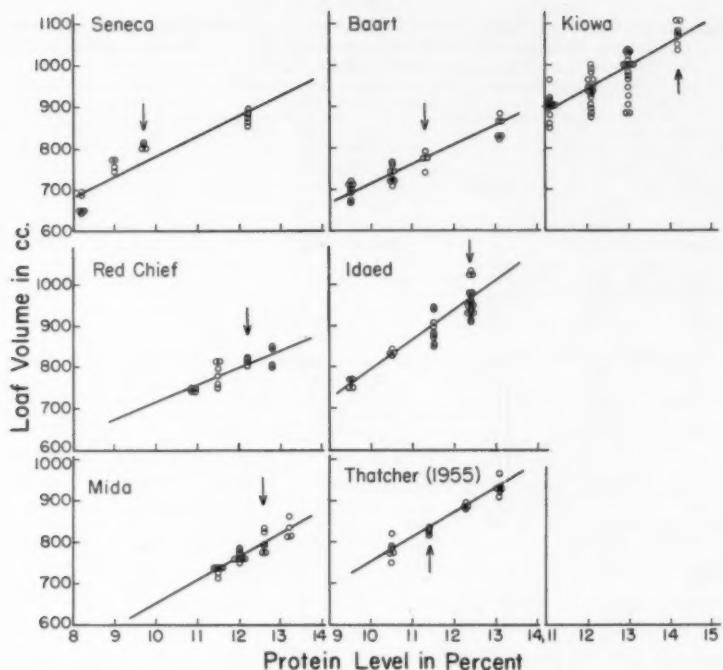


Fig. 4. Loaf volume values obtained by enrichment and dilution of flours with their own components. Small vertical arrows indicate natural protein content of each of the flours.

reconstituted doughs was very small in the absence of solubles fractions.

In order to investigate the value of this method for assay of baking quality of the total protein components of flours, glutens and solubles fractions from a number of flours were added at several levels to a low-protein base flour (Yorkwin variety, 7.6% protein). As in each instance when these proteins were added back to flours and doughs, each increment of gluten was accompanied by an exactly proportionate increment of the total solubles from the same source. The baking results are shown in Fig. 2. The regression equations obtained are compared graphically with one another in Fig. 3 and with results obtained by enrichment and dilution of the flours with their own components. The latter are shown in detail in Fig. 4. Numerical values are given in Table II and statistical evaluation of the relationships is illustrated in Table III. The equations for the enriched and diluted flours were calculated from loaf volume values obtained at protein levels ranging

TABLE II  
REGRESSION EQUATIONS OBTAINED BY ENRICHMENT AND DILUTION OF FLOURS WITH  
THEIR OWN PROTEIN AND STARCH COMPONENTS AND BY ADDITION OF PROTEIN  
COMPONENTS TO A LOW-PROTEIN BASE FLOUR (YORKWIN VARIETY)

VARIETY	ENRICHMENT AND DILUTION <sup>a</sup>	ENRICHMENT OF BASE FLOUR <sup>b</sup>
Seneca	48.6 X + 296	40.8 X + 329
Baart	41.6 X + 302	56.4 X + 187
Thatcher (1955)	57.9 X + 172	55.0 X + 213
Red Chief	41.5 X + 302	33.4 X + 401
Idaed	70.2 X + 93	60.0 X + 137
Mida	56.9 X + 81	51.5 X + 241
Kiowa	52.6 X + 306	59.0 X + 173

<sup>a</sup> LSD between slope values is 14.9 at 5% point.

<sup>b</sup> LSD between slope values is 12.8 at 5% point.

TABLE III  
VARIANCES FOR LINEAR COMPONENTS AND DEVIATIONS FROM REGRESSIONS OF LOAF  
VOLUME ON FLOUR PROTEIN IN TABLE II

VARIETY	ENRICHMENT AND DILUTION			ENRICHMENT OF BASE FLOUR		
	Linear Component	Deviation	F Ratio	Linear Component	Deviation	F Ratio
Seneca	124,086	1,245	99.6**	12,869	245	52.6**
Baart	80,613	407	198.0**	34,833	356	98.0**
Thatcher (1955)	73,498	245	300.0**	22,385	77	291.4**
Red Chief	14,465	460	31.4**	9,902	149	66.3**
Idaed	158,103	985	160.5**	32,925	196	168.4**
Mida	26,608	310	85.9**	20,244	210	96.2**
Kiowa	129,585	1,645	78.8**	30,848	77	400.0**

from 8 to 14% protein. This means that the lines for all but Kiowa and Idaed flours were obtained for points falling above and below their original protein content and in a region where the line for Seneca in Fig. 1 appears to be undergoing rapid inflection. Nevertheless, in spite of possible wide deviations from linearity, the regression for Seneca was still highly significant.

A pronounced but incomplete shift of the lines toward a common convergence resulted from additions of the various proteins to the base flour. However, a decrease in slope values occurred for all flours except Kiowa and Baart (Table II). For these, modest increases occurred. The decrease in value for Thatcher flour was very small. It is possible that differences in blending or supplementing value of the added proteins were responsible for the changes in slope values. Despite the great improvement in vertical scatter of the lines due to use of a common starch system, the use of a base flour for comparison of proteins from different flours seems questionable because of the apparently random alteration of the slope values of most of the lines, but primarily because a mixture of protein is being evaluated.

*Interchange of Starch and Protein Components in Reconstituted*

TABLE IV  
PROTEIN LEVELS (14% MOISTURE) AND AVERAGE LOAF VOLUMES FOR VARIOUS COMBINATIONS OF STARCHES AND PROTEINS FROM THREE DIFFERENT FLOURS IN  
TOTALLY RECONSTITUTED DOUGHS

SOURCE OF PROTEIN	SOURCE OF STARCH					
	Seneca		Red Chief		Thatcher	
	%	cc	%	cc	%	cc
Seneca	9.5	661	9.6	635	9.5	690
	10.2	699	10.3	685	10.2	730
	10.8	738	10.8	723	10.8	740
	11.6	791	11.5	773	11.5	781
Red Chief	10.0	743	10.0	662	10.0	661
	10.5	750	10.7	689	10.7	694
	11.3	796	11.4	731	11.4	724
	12.0	822	12.1	755	12.1	773
Thatcher	11.3	769	10.0	714	10.0	733
	12.0	824	10.7	773	10.7	740
	12.6	864	11.3	823	11.3	821
	13.3	888	12.0	841	12.0	853

*Doughs.* Another method of comparing the baking performance of flour proteins independently of differences in starch composition is to combine proteins from each flour with starch from a single source in completely reconstituted doughs. This method is quite laborious, subject to some uncertainty as to proper recombination of dough components, and possibly subject to errors due to differences in any interaction effects between the starch selected and the protein components of the different flours.

In an effort to obtain the maximum amount of information on these points with as simple an experiment as seemed possible, starch and protein components of three different flours were combined in all combinations. Thus, the proteins (gluten plus total solubles) of each flour were baked with the starch from each flour in turn. Great care was taken to bake each combination of components as near as possible to its optimal conditions for maximum loaf volume production, with particular attention to mixing time, bromate level, and water absorption. With Red Chief proteins and Seneca starch it was found necessary to use the starch within a few days after it was isolated to avoid serious discrepancies in loaf volume results for any given level of recombination of components. No explanation can be offered for this pronounced sensitivity of Red Chief proteins to the age of Seneca starch. It was not encountered with starches from either of the other flours or with Seneca starch combined with either of the other proteins. No correlation was found between this behavior and total lipids, free fatty acids, or peroxide values of Seneca starch preparations.

TABLE V  
REGRESSION COEFFICIENTS FOR LOAF VOLUME ON PROTEIN LEVEL (AT 14% MOISTURE)  
FOR COMBINATIONS OF STARCHES AND PROTEINS FROM  
THREE FLOURS IN RECONSTITUTED DOUGHS\*

SOURCE OF PROTEIN	SOURCE OF STARCH		
	Seneca	Red Chief	Thatcher (1955)
Seneca	61.9	72.5	43.5
Red Chief	42.1	45.6	52.1
Thatcher (1955)	59.9	65.4	73.7

\* Over-all linearity of all the regressions is significant at the 5% point when tested against deviations from linear regression.

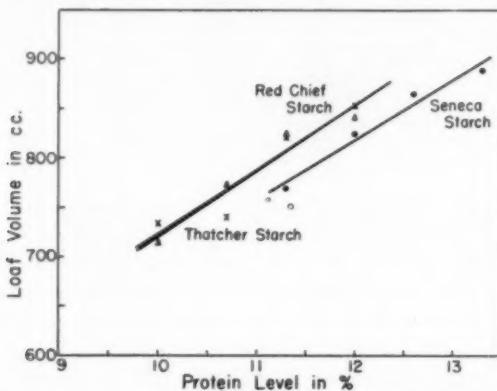


Fig. 5. Regression lines obtained with Thatcher (1955) proteins and starches from Seneca, Red Chief, and Thatcher (1955) flours in completely reconstituted doughs.

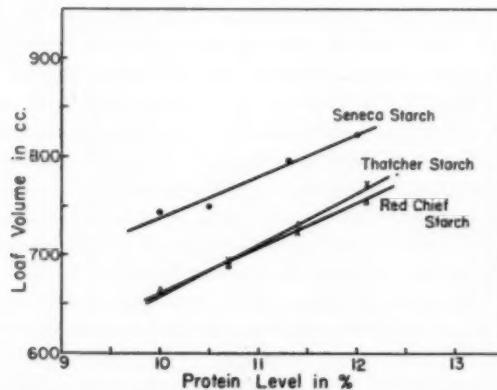


Fig. 6. Regression lines obtained with Red Chief proteins and starches from Seneca, Red Chief, and Thatcher (1955) flours in completely reconstituted doughs.

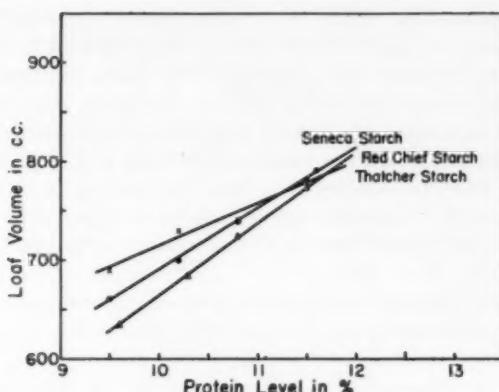


Fig. 7. Regression lines obtained with Seneca proteins and starches from Seneca, Red Chief, and Thatcher (1955) flours in completely reconstituted doughs.

The results of these experiments are summarized in Table IV and Figs. 5, 6, and 7. Regression coefficients for the nine combinations of ingredients are given in Table V.

The most nearly uniform results with respect to slope values for the three starches were obtained with Thatcher and Red Chief proteins. The majority of the slope values agree reasonably well with those obtained by enrichment and dilution of Thatcher and Red Chief flours with their own components (Table II). It is of interest to note, however, that Red Chief and Thatcher starches with Thatcher proteins gave a line situated definitely above that for the Seneca starch (Fig. 5). With Red Chief proteins, on the other hand, Red Chief and Thatcher starches gave lines placed very much lower than that for Seneca starch (Fig. 6), which was the only one of the three to give loaf volumes with Red Chief proteins equal to those for Thatcher proteins as shown in Fig. 5. This behavior with Red Chief and Thatcher starches seems to be clear evidence of an interaction effect. Slope values for Seneca proteins varied the most, and there was a tendency for the lines to converge near 12% protein (Fig. 7). At this protein level, however, loaf volumes are nearly as large as those obtained with Thatcher proteins.

It is unfortunate that at least one complete replication of this entire experiment was not possible to provide a measure of the variability of the regression coefficients shown in Table V. However, the over-all linearity of the regressions due to protein level for each flour is highly significant when tested against individual deviations from linear regression. Although no systematic effort to randomize effects of variables was

made, the manner in which these experiments were conducted furnished a reasonable approach to freedom from systematic errors. Gluten and other fractions were prepared from flours in relatively small batches, usually from about 2 kg. of flour. Replicate bakes for any one combination of starch and proteins were always conducted on different days and often with different batches of glutens and their accompanying water-solubles or starches. Thus partial replication of the experiments was provided, together with confidence that complete replication of the entire experiment would give results in agreement with those presented.

Much of the information just presented strongly suggests significant effects due to starches or to starch-protein interactions. An analysis of covariance was performed on the data shown in Table IV in order to try to verify these effects, and results of the analysis are given in Table VI. Before covariance the only significance was for starch sources, which indicates differences in mean values for the different starch sources. After adjustments for variation in protein levels, starch sources still had significance. Protein source and interaction of starch and protein sources also were significant after covariance adjustments were made. This indicates that after the variation that existed in protein levels was taken into account, there were significant differences between starch sources and protein sources, and there was significant interaction of these two factors.

An interpretation of the meaning of the results of this experiment might proceed as follows: the tendency for slope values to be constant, as with Thatcher and Red Chief proteins when combined with different starches, can perhaps be regarded as principally a protein effect. These two proteins apparently have good tolerance to starches in general. The differences in vertical placement of regression lines of reasonably uniform slope can perhaps be regarded as principally a starch effect. If lines vary in vertical placement as well as in slope, when different combinations of starch and proteins are used, the effect seems

TABLE VI  
ANALYSIS OF VARIANCE AND OF COVARIANCE FOR LOAF VOLUME,  
WITH PERCENT PROTEIN AS THE INDEPENDENT VARIABLE

SOURCE	D. F.	MEAN SQUARE	ADJUSTED MEAN SQUARE
Flours	8	8532**	3934**
Starch source	2	24005**	6331**
Protein source	2	6600	1531**
Starch source $\times$ protein source	4	1760	3393**
ERROR	27	2535	170*

\* This error is based on 26 d. f., since the error line was used for the regression line.

due to interactions between starch and protein, with protein perhaps being the factor most susceptible to influence.

### Discussion

Objectives of the experimental work reported here seem to be only partly achieved. That starches of different flours or different classes of flours are responsible for the sometimes large differences in vertical placement of protein-loaf volume regression lines seems fairly well established. More confidence thus can be placed in the view that regression coefficients, obtained by fortification and dilution with a flour's own components, are the best estimates yet available for research purposes of *protein* quality (for loaf volume production) of individual flours. Much uncertainty remains, however, with respect to the extent that starch-protein interaction effects may influence the overt behavior of proteins of any given flour. This uncertainty will remain until additional work can evaluate its limits. It is possible, for example, that such interaction effects are to be found principally with soft wheats and would not be of significant magnitude in hard wheat classes.

The method for evaluation of baking quality (for loaf volume production) of different *flours* proposed by Finney and Barmore (6) seems entirely applicable, at least for research and variety evaluation, so long as comparisons are limited to varieties within a given class, such as hard red winter and spring classes. Durum flours and possibly a few varieties such as Red Chief, Chieftan, etc., probably should be omitted because of the tendency for their regression lines to converge at a different protein level. A separate array of idealized regression lines probably should be set up for them. Soft wheat flours may also need to be set up in a separate class; much work needs to be done to see how well a large number of samples of this class will converge to a common point, perhaps different from that found for hard red wheats, and to what extent occasional samples or varieties may deviate from the general behavior observed. As a matter of fact, considerable effort in this direction has already been made (2). Even so, it will always be desirable to make interclass comparisons of baking qualities, even if only for research purposes. No completely satisfactory solution to the problem appears imminent. The method used in the present paper, wherein regression values are obtained by protein adjustment of individual flours, appears to be the most satisfactory for comparison of *proteins* from different flours. Until more information is available, however, the method will always be subject to an unknown degree of uncertainty regarding the extent to which the baking behavior of a given protein system will be influenced by the starch with which it may be combined.

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## HYDROXYETHYLATED CEREAL FLOURS<sup>1</sup>

J. C. RANKIN, C. L. MEHLTRETTTER, AND F. R. SENTI

### ABSTRACT

Flour in a dry mixture with alkali catalyst was readily hydroxyethylated with ethylene oxide. Cooked pastes of etherified flours were fluid in character, did not retrograde on cooling and aging, and had a pattern of viscosity behavior in the cooking-cooling cycle comparable to that of similarly substituted starches. Their clarity was not equal to that of hydroxyethyl starch pastes; however, reacting flour or starch with ethylene oxide significantly improved paste clarity of either starting material. Surface tension measurements showed that the modified flour pastes had unusual surface activity.

Quantitative yields of hydroxyethylated flours obtained by this relatively simple procedure, together with improved viscosity properties of cooked pastes over those of unreacted flour, indicate promise for these cereal products in industrial applications.

Wheat flour is a relatively well refined raw material that is available in abundance and at lower cost than either of its main constituents, starch and protein. The latter substances have found wide commercial use, but the use of cereal flours in the nonfood industries has been small. It is believed that expanded utilization of cereal flours will follow economical modification of their physical and chemical behavior by chemical reaction to meet the range of properties required for varied purposes.

Significant changes have been made in the properties of starch and of cellulose by etherification of these polysaccharides with ethylene oxide (4,6,7,8,12), and such modification has greatly increased their utility (2,10). It was therefore considered desirable to hydroxyethylate cereal flours, which are comparable materials, and to determine the pasting properties of the products obtained as a basis for industrial evaluation.

Hydroxyethylation of flour in an aqueous medium was found impractical. A gummy residue was produced which was difficult to isolate in good yield. Satisfactory etherification was achieved by reacting dry flour with gaseous ethylene oxide in the presence of an alkaline catalyst. Practical advantages obtained by dry reaction of flour with ethylene oxide were: 1) preventing loss of product through solution, and 2) simplifying product recovery by eliminating filtering and drying operations. It is presumed that both the starch and protein in flour were hydroxyethylated since each was etherified separately under the conditions employed.

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### Preparation of Hydroxyethylated Cereal Flours

Commercial cereal flours used in this study ranged in starch content from 65 to 89% and in protein content from 7 to 20% and had the usual moisture content. In occasional samples, the protein and starch values varied from these limits. Corn starch, wheat starch, and wheat gluten were hydroxyethylated for purposes of comparison.

Ethylene oxide and other compressed gases used were commercial products. Their purity was listed as 99.5%. The inorganic salts and alkali used were of reagent grade.

*Hydroxyethylation Procedure.* A commercial wheat flour of approximately 14% moisture content was mixed thoroughly with 2.5% of dry, powdered sodium hydroxide. After aging at room temperature for 4 days the mixture was placed in a jacketed laboratory kneading machine provided with a combination vacuum-pressure gage. Air was removed from the vessel to obtain 28 in. of vacuum, and gaseous ethylene oxide was then introduced to achieve atmospheric pressure. Mixing was begun, and additional quantities of ethylene oxide were added at intervals when the pressure had dropped as a result of the gas reacting with the flour. A total of 12% ethylene oxide<sup>2</sup> was introduced over a period of 8 hours. The reaction was maintained at approximately 43°C. by intermittent passage of steam through the jacket of the mixer.

After removal from the mixer, the product was a finely divided free-flowing powder, much like the original flour in appearance. It was obtained in quantitative yield and had a moisture content of about 10%. The amount of ethylene oxide that reacted was approximated from the pressure drop measured by gage readings and then determined accurately by analysis of the sample for hydroxyethyl content by the method of Lortz (9). All ethylene oxide values reported in this paper were determined by analysis and were calculated as percentages of ethylene oxide in the final product on the dry, ash-free basis. Dialysis and extraction (acetone) experiments to remove any glycol and polyoxyethylene by-products gave products which analyzed within 0.5% of the original ethylene oxide content. This was well within limits of experimental error for the method of analysis. The alkalinity of modified flour can be reduced by treatment in the reaction vessel with carbon dioxide to pH 9.5, powdered boric acid to pH 9, or hydrogen chloride gas to pH 4-7. Conditions for reaction of ethylene oxide with various cereal flours and pertinent analytical data from experiments are given in Table I.

<sup>2</sup> Twelve percent of the combined weight of flour and of ethylene oxide that had reacted.

TABLE I  
HYDROXYETHYLATION OF CEREAL FLOURS

FLOUR	REACTION CONDITIONS		PROTEIN CONTENT OF FLOUR <sup>a</sup> (Percent of Original)	ANALYSIS OF PRODUCT <sup>b</sup>		
	TEMPERATURE °C	TIME Hours		Moisture	Ash	C <sub>2</sub> H <sub>5</sub> O
Hard winter wheat	31	2	13.49	12.77	1.95	2
Hard winter wheat	37	6	13.49	11.81	2.26	7
Hard winter wheat	43	8	13.49	9.91	2.03	12
Hard winter wheat	36	9	13.49	11.36	2.07	15
Hard winter wheat	46	14	13.49	7.65	2.38	16
Hard winter wheat	34	40	13.49	8.92	1.81	30
Hard winter wheat, second clears	40	9	13.96	10.00	2.78	12
Soft white wheat	43	8	7.70	9.91	2.03	12
Soft red winter wheat	31	8	9.97	9.21	2.43	11
Rye	40	12	10.31	10.25	2.66	13
Oat	36	12	19.63	8.51	3.33	16
White corn	36	7	8.88	11.08	2.13	16
Yellow corn	37	10	7.44	11.11	2.04	19
Wheat gluten	51	5	75.00	5.48	2.85	8
Wheat starch	39	10	0.25	9.31	1.43	12
Corn starch	39	8	0.31	8.91	2.24	12

<sup>a</sup> Kjeldahl nitrogen, N × 5.7 for percentage protein in wheat flour, N × 6.25 for the other cereal flours.  
Protein content was corrected for ash and moisture present.

<sup>b</sup> Values for C<sub>2</sub>H<sub>5</sub>O are on a dry ash-free basis and were obtained by analysis (9).

Batches of 125 g. of flour could readily be reacted with ethylene oxide in a 1-qt. sigma-blade kneading machine. As shown in Table I, the separate constituents of wheat flour, starch and gluten, also were easily etherified by this dry reaction procedure. Most of the experiments were made at atmospheric pressure or below for safety reasons. In general, the reactions were controlled within a temperature range 25°–45°C. to avoid swelling or gelatinizing of the starch in the flour. Control runs (flour and alkali) were made in an atmosphere of nitrogen instead of ethylene oxide.

Optimum reaction conditions were obtained by using 2.5% sodium hydroxide based on the combined weight of flour and alkali. Less basic substances such as the alkali carbonates, borates, and chlorides were also effective when used at 1–5% concentrations.

#### Evaluation of Hydroxyethylated Cereal Flours

Properties desired in starch and modified starches for various industrial applications have been stated by Schoch (11). Important criteria for judging the utility of a starch product are its paste viscosity and paste clarity. The stability of such pastes in regard to viscosity and clarity on heating, cooling, and aging is also important. A moderate degree of penetration may also be desirable and can be estimated by surface tension measurement of the pasted products. The

hydroxyethylated (HE) flours would be required to possess these characteristics for industrial uses as outlined.

*Viscosity.* The Corn Industries Viscometer (5) which was used for measuring the viscosity behavior of the modified pastes was operated at a stirrer speed of 60 r.p.m. A temperature of 92°C. was maintained in the water bath. Paste viscosity was measured in g.-cm., but is also reported in centipoises (5). Products tested were added to distilled water and stirred mechanically to obtain a smooth paste which was then poured into the preheated viscometer. Viscosity was generally determined at pH 10-11, the normal pH of the paste, which was measured before and after cooking. The effect of pH 4 and 7 on viscosity behavior of the pasted products was also determined. The cooking-cooling cycle used in the viscosity studies consisted of heating the paste at 92°C. for 30 minutes, then reducing the temperature to 25°C. by cooling for 30 minutes with cold water admitted to the bath from a constant head.

Illustrated in Fig. 1 are viscosity patterns of 8% pastes of hard red winter wheat flour, HE flour, and a control flour containing the catalyst but not hydroxyethylated. Gelatinization temperature is recorded

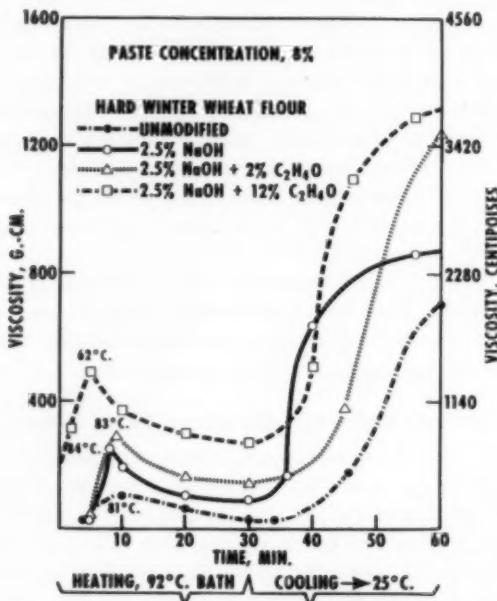


Fig. 1. Viscosity of pasted flour and hydroxyethylated flour.

on each curve. Hydroxyethylation decreased the temperature and time of gelatinization of the flour and increased its paste viscosity. During the cooking cycle the viscosity was quite stable and was not lowered appreciably over a period of 2 hours at 92°C. The hydroxyethylated flour pastes thickened on cooling, but showed little tendency to retrograde.

Although unmodified flour and starch are quite different in rheological properties, after hydroxyethylation their pastes gave comparable viscosity patterns (Fig. 2). The etherified flours had somewhat lower paste viscosities than their starch counterparts. This difference may be attributed partially, at least, to dilution of starch content in the flour by its protein and other components. This relationship is borne out by comparison of paste viscosities of HE flours, as shown in Fig. 2 and again in Fig. 3 for high- and low-protein etherified flours.

Figure 4 shows that the difference in maximum hot paste viscosities of HE flour over the pH range 4–11 was about 100 g·cm. This differ-

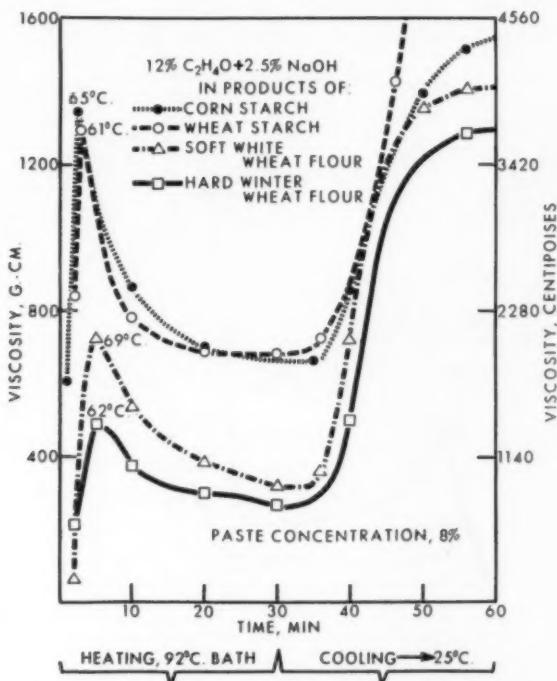


Fig. 2. Viscosity of pasted hydroxyethylated starch and flours.

ence was even less for the corresponding flour treated with alkali catalyst. The maximum hot paste viscosity curve for the unmodified flour between pH 7 and 11 was nearly the same as that for the alkali-treated flour. Upon acidification to pH 4 with hydrochloric acid, however, the maximum hot paste viscosity of the unmodified flour rose to over threefold its value at neutrality, a difference of approximately 475 g.-cm.; whereas that of the alkali-treated flour, whether hydroxyethylated or unmodified, was only slightly affected. Gelatinization temperatures of the flour pastes within these pH limits did not vary as much as those for the HE flour. Figure 5 shows the effect of pH on the pasting properties of 12% HE flour. Eight percent aqueous pastes produced under alkaline and acid conditions gave higher maximum hot paste viscosities than a similar neutral paste. The lower "set-back" viscosity found at 25°C. for the alkaline and acid pastes can be attributed to slight degradation of the product during the cooking period.

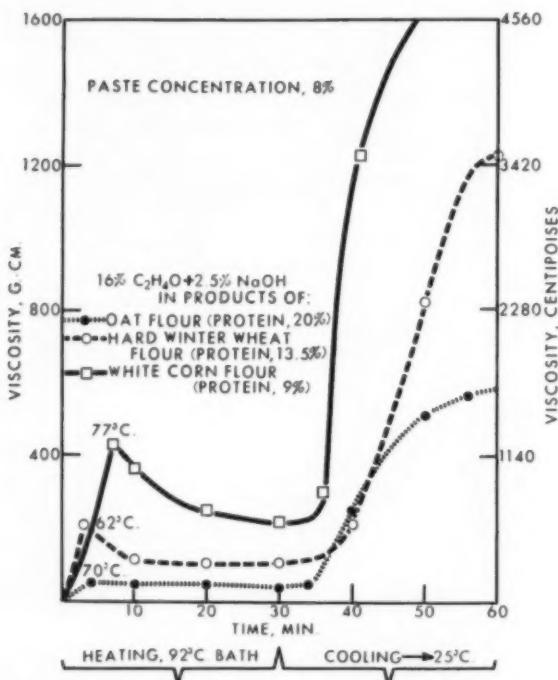


Fig. 3. Effect of protein content of hydroxyethylated flours on paste viscosity.

A study is now in progress to determine the effect of storage of hydroxyethylated flours under acid, neutral, and alkaline conditions on the viscosity and clarity of their pastes.

The influence of ethylene oxide content on maximum hot paste viscosity of HE hard winter wheat flours is illustrated in Fig. 6. Gelatinization time and temperature were reduced with increased substitution of ethylene oxide. Greatest viscosity was obtained with flour containing 12% ethylene oxide.

Hydroxyethylated flours produced cooked pastes with maximum cold viscosities ( $25^{\circ}\text{C}.$ ) above those found with pastes of unmodified flour or a control of alkali-flour. This property varied only slightly over a wide range of hydroxyethyl content.

As expected, the maximum hot paste viscosity of HE flour increased with concentration, whereas clarity of the pastes decreased (Fig. 7). Only slight viscosity was obtained below 5% concentration.

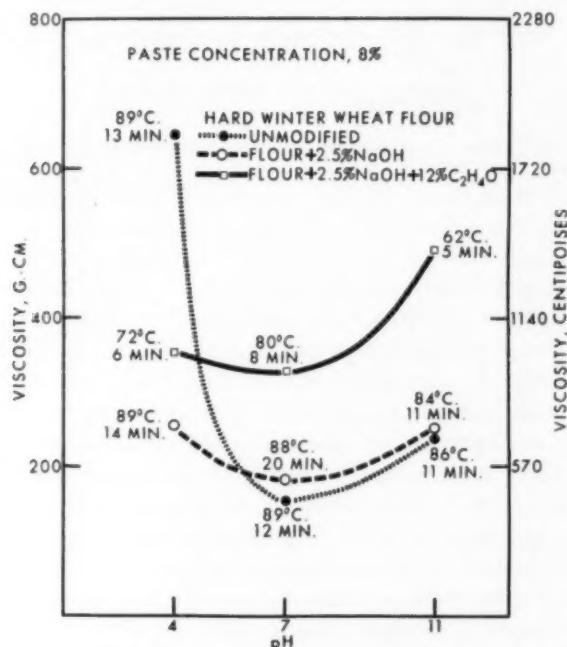


Fig. 4. Effect of pH on maximum hot paste viscosity of flour and hydroxyethylated flour.

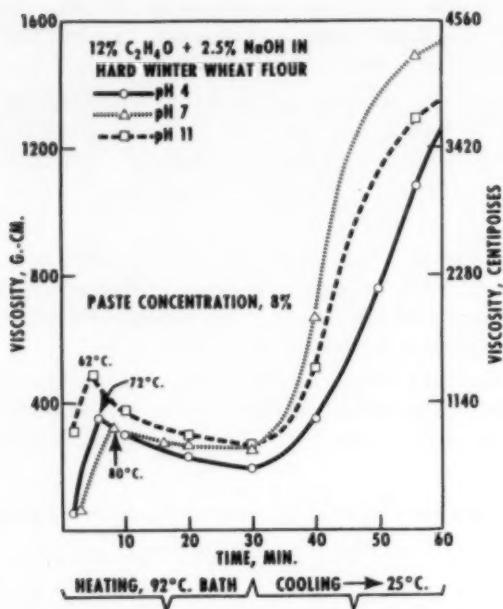


Fig. 5. Effect of pH on paste viscosity of hydroxyethylated flour.

**Clarity.** Paste clarity was determined as follows: Weighed samples of products were dispersed in 20 ml. of distilled water in matched test tubes (Pyrex 9800, 18 by 150 mm.) by cooking for 30 minutes with stirring in a boiling-water bath followed by cooling for 1 hour in running tap water. Water was added when necessary to compensate for loss during heating and the pastes were stored for 24 hours at 8°C. After the pastes had been allowed to adjust to room temperature, readings were made in a Coleman<sup>3</sup> Spectrophotometer. Paste clarity was reported as percentage transmission of light at 650 m $\mu$ , based on distilled water as the standard for 100% transmission. The pH of the cooked solutions was then measured and in some instances pH of the paste was adjusted to 4, 7, or 11 with either hydrochloric acid or sodium hydroxide, and the percentage transmission of light was again determined. Clarity measurements also were made on the pastes after 48 hours' standing at room temperature.

<sup>3</sup> Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

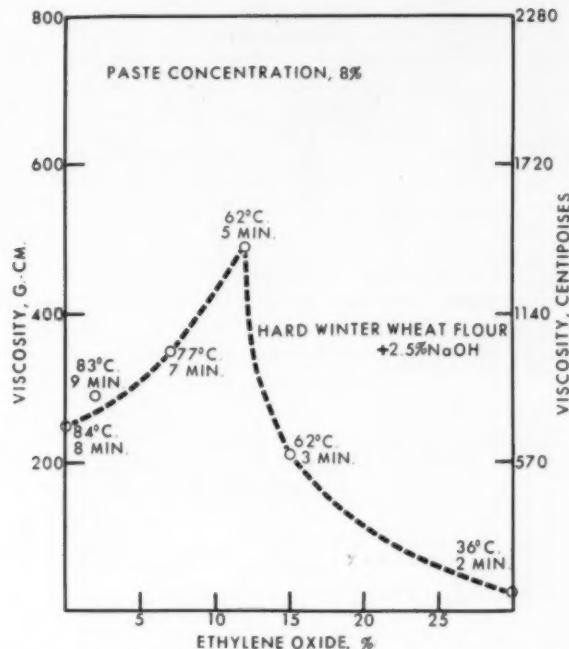


Fig. 6. Effect of ethylene oxide content on maximum hot paste viscosity of hydroxyethylated flours.

As shown in Table II, paste clarity of the starches and flours was improved by etherification — however, pastes of HE flours did not attain the clarity of those of HE starch. A similar variation in clarity was noted for pastes of unmodified flour or starch with and without alkali addition; apparently this property was carried through to the etherified products. Paste clarity is greatest at pH 11 for the unmodified and substituted products. It is significantly reduced for the HE flour at lower pH values, as seen in Fig. 8. Pastes of 5% concentration of HE products were clearer because of dilution than those of 8%. Clarity is also influenced by ethylene oxide content, as shown in Fig. 9 for a hard winter wheat flour. In 1 and 5% pastes an ethylene oxide content of about 12 % produced the best clarity. To achieve maximum clarity with greatest maximum hot paste viscosity in a 12% HE flour, a paste concentration of 8% was required (Fig. 7).

TABLE II  
PASTE CLARITY DATA

SAMPLE	PASTE CLARITY <sup>a</sup>					
	1% Paste		5% Paste		8% Paste	
	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours
Corn starch	50	45	5	5	5	5
Corn starch <sup>b</sup>	84	79	21	21	7	7
Corn starch <sup>c</sup>	85	85	82	82	78	78
Wheat starch <sup>c</sup>	81		43			
Hard winter wheat flour	12	10	3	3	2	2
Hard winter wheat flour <sup>b</sup>	58	55	4	4	1	1
Hard winter wheat flour <sup>c</sup>	78	78	53	53	19	19

<sup>a</sup> Percent transmission of light.

<sup>b</sup> Contains 2.5% NaOH, the concentration of catalyst used in the hydroxyethylation.

<sup>c</sup> Contains the catalyst and 12% C<sub>2</sub>H<sub>4</sub>O.

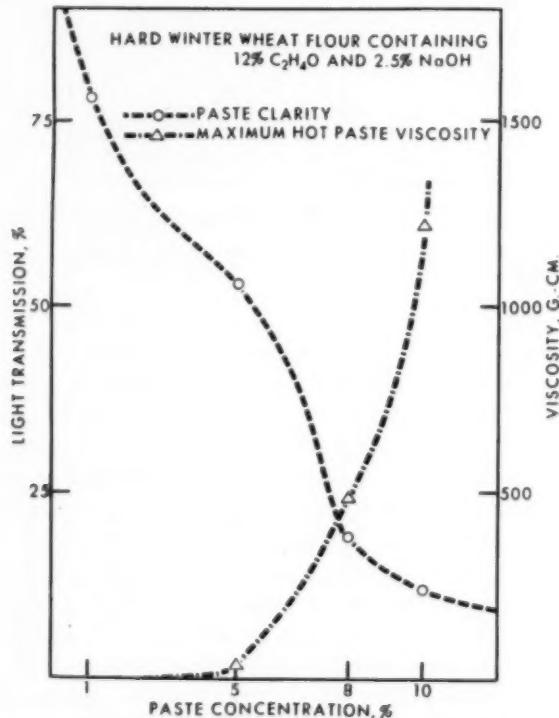


Fig. 7. Effect of concentration on paste viscosity and clarity of hydroxyethylated flours.

TABLE III  
SURFACE TENSION OF HYDROXYETHYLATED PRODUCTS

PRODUCT *	1% Paste	5% Paste
	dynes/cm	dynes/cm
Hard winter wheat flour	40.7	43.2
Hard winter wheat flour, second clears	40.3	40.1
Soft white wheat flour	42.5	44.4
Wheat starch	55.7	61.9

\* Contains catalyst and 12%  $C_2H_4O$ .

*Surface Activity.* Surface tension of the pastes from HE products was measured with a Du Noüy interfacial tensiometer at 25°C. by the ring method. Benzene was the standard liquid used (3). Pastes were prepared as described for the clarity tests. Table III shows that the 12% HE flours have marked surface activity.

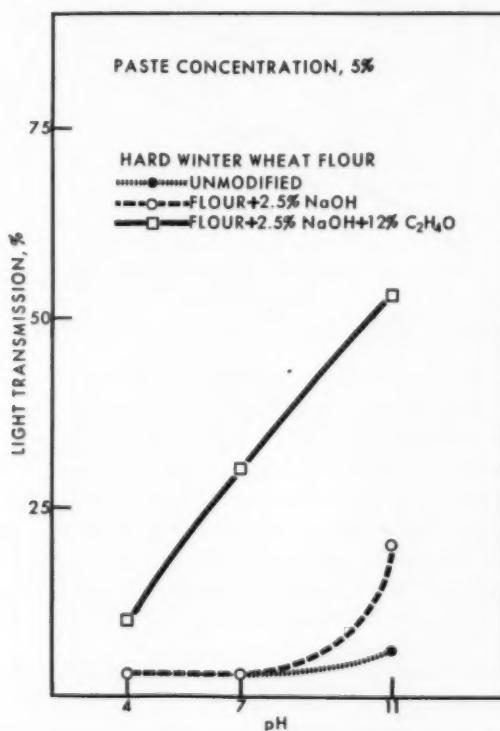


Fig. 8. Effect of pH on paste clarity of pasted flour products.

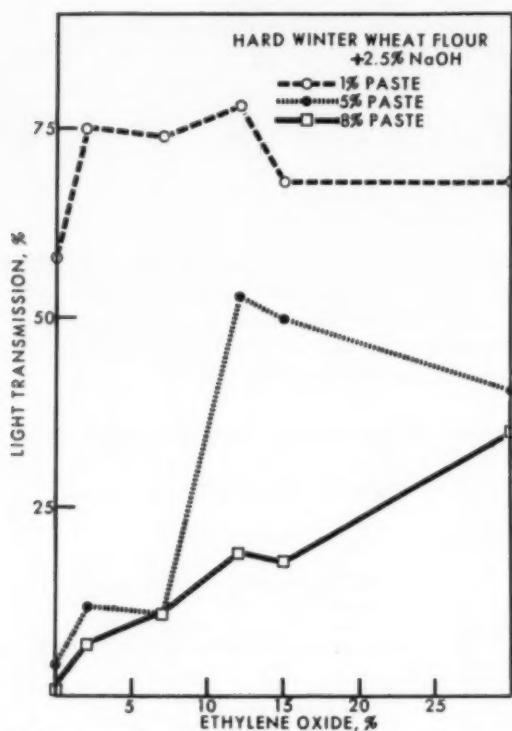


Fig. 9. Effect of ethylene oxide content on paste clarity of flour products.

#### Summary and Conclusions

Although it is theoretically possible to react all of the hydroxyl groups of the starch in flour with ethylene oxide, a low degree of substitution effects as pronounced changes in the viscosity behavior of pastes of flour as it does in that of starch pastes. Such substitution apparently reduces the tendency of the hydrated starch molecules in cooked pastes to associate with each other and to retrograde upon cooling (10). It also modifies the glutinous character of flour pastes, thus making it possible to achieve simultaneous dispersion of gluten and starch in the flour (1).

Hydroxyethylation lowers the time and temperature of gelatinization and imparts a greater viscosity to flour pastes. Paste viscosity, which may be varied by changing the degree of substitution of ethylene oxide, is relatively stable to differences in pH and cooking time. Cold pastes

have little tendency to gel or to retrograde. Ethylene oxide treatment brings about a marked improvement in the paste clarity of native flour. The fact that the modified flours have considerably greater surface activity than hydroxyethylated (HE) wheat starch suggests that this property is derived from HE gluten and from small quantities of HE fatty acids in the products.

The facile procedure developed for hydroxyethylation of cereal flours in the dry state produces free-flowing products in quantitative yield which have pasting properties superior to those of unmodified flours. These results indicate that HE flours have potential value as low-cost sizing agents for application in the paper, textile, and allied industries.

#### Acknowledgment

The authors are indebted to Mr. J. G. Rall for his technical assistance during portions of the investigation and to Mrs. Clara E. McGrew for ethylene oxide, ash, and nitrogen determinations.

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## SOME FACTORS THAT AFFECT THE STALING OF WHITE AND YELLOW LAYER CAKES<sup>1</sup>

K. KULP, J. G. PONTE, JR.,<sup>2</sup> AND W. G. BECHTEL

### ABSTRACT

Oxidative changes decreased the freshness of cakes stored at 70°-72°F. (21°-22.5°C.). Canned cakes stored under nitrogen to prevent oxidation and those wrapped in metal foil to prevent loss of moisture staled appreciably during the first few days of storage. During this period the cakes firmed rapidly. One-half of the total firming occurred in the first 4 days. Between one-third and one-half of the staling observed by the panel in the 27-day test period occurred within the first 4 days. It thus appears that loss of freshness in the first few days of storage is related to changes in the physical characteristics of the cake. Moisture loss from cakes was interpreted by the panel to cause rapid staling.

The term cake staling, as used in this paper, is defined as consisting of all the changes which result in decreased consumer acceptance of the product, other than those which result from the action of spoilage organisms. This definition follows from the discussion of Bice and Geddes (5) and the later work of Bechtel, Meisner, and Bradley (4) on bread staling. It implies that staling will be determined by consumer evaluation of the product. Such evaluation is made in the laboratory by sensory panel tests.

While bread staling has been investigated extensively, the literature contains only a few reports of studies of the changes in cake properties during storage. In 1933 Platt and Kratz (10) reported that during storage for 36 days the compressibility and elasticity of the crumb of sponge cake decreased and the tensile strength increased. Noznick and Geddes (8) found that firmness of sponge cake increased during storage at a greater rate than that of yellow loaf cake. Staling of pound cake was studied by Bechtel and Meisner (3), who found that moisture-proof packaging retarded staling appreciably and that cakes in vacuum-sealed tinned cans staled very little during a storage period of 60 days.

The possibility that oxidative changes might be a factor in cake staling is suggested by the studies of Meyer *et al.* (7), who observed flavor deterioration of egg-yolk lipids during freezer storage of cake batter. Oxidative changes were considered by Platt and Kratz (10) to be a probable factor in staling of sponge cake, although they made no experimental studies to test the hypothesis.

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<sup>2</sup> Present address: Continental Baking Company, Rye, New York.

The object of the present study was to ascertain the relationship of moisture loss, oxidation, and changes in firmness and crumbliness to the staling of white and yellow layer cakes.

### Materials and Methods

*Preparation of Experimental Cakes.* Yellow and white layer cakes were prepared according to the formulas given below. They represent types currently used in commercial cake production. To simplify the flavor profile of the product and render the detection of off-flavors easier for the taste-test panel, no added flavor was used.

Ingredient	Yellow cake		White cake	
	parts by wt.		parts by wt.	
Flour	100.0		100.0	
Shortening (superglycerinated type)	35.0		36.0	
Sugar	120.0		120.0	
Salt	3.10		3.5	
Baking powder (pyrophosphate type)	6.25		5.0	
Nonfat dry milk	7.50		8.0	
Water	75.0		89.0	
Egg, whole (frozen)	40.0		—	
Egg whites (frozen)	—		47.0	
Acid salt (cream of tartar)	—		0.5	

Twelve ounces of yellow and 14 oz. of white batter were used for conventional layer cakes of 8-in. diameter. For canned cakes, tinned cans without enamel, 6 in. in diameter and 2 1/4 in. high, were used. They were lined on bottom and sides with vegetable parchment. The amount of batter, 8 oz., was sufficient so that the center of the cake just touched the lid. Because products baked in cans covered during baking were of poor quality as judged by flavor, texture, and volume, lids were placed on the cans for only the last few minutes in the oven. This method produced cakes which were comparable in quality to conventional layers.

*Sealing Cans and Filling with Oxygen or Nitrogen.* The cans were sealed with a manual sealer as rapidly as possible after removal from the oven, to produce maximum vacuum. After a cooling period of approximately 3 hours some of the cans were filled with oxygen or nitrogen. To do this, the cans were punctured with a vacuum gage and the vacuum was measured. Only cans with a vacuum above 25 inches were used for further experiments. With the gage in place, cans were placed in an atmosphere of oxygen or nitrogen. A vacuum desiccator was adapted as a gas chamber for this purpose. The outlet was connected by means of a three-way stopper with a gas cylinder (oxygen or nitrogen) and a vacuum pump which produced a 28-inch vacuum in this

chamber. The gas chamber was evacuated and then tilted to remove the gage from the can, after which it was filled with the desired gas. Evacuation and filling were repeated two additional times. The can was then removed from the chamber and the opening sealed with paraffin when oxygen was used, or soldered with tin in the case of nitrogen. Pure oxygen was employed rather than air, because the space for gas was limited; thus more extensive oxidative changes were permitted.

*Cake Storage.* Cakes were kept in a cabinet automatically held at 70°–72°F. (21°–22.5°C.), except for those used to determine the effect of moisture loss. These cakes were kept in a laboratory drawer at 70°–78°F. (21°–25.5°C.).

*Panel Selection.* The method of training and selection of the panel was that used by Bechtel and Meisner (1) for the study of bread staling.

*Testing Method.* The testing procedure was essentially that used by Bechtel and Meisner (2) in bread staling studies. Samples were prepared as follows: the edges and crust of the cake were removed. Slices  $\frac{1}{2}$  in. by 1 in. by 2 in. were cut and placed immediately in moisture-proof cellophane bags which were heat-sealed. Each sample was coded with a two-digit number selected by chance, and each bag carried the sample number. Panel members were directed to open one sample bag at a time and judge the freshness by feel in the fingers; by odor, flavor, feel in the mouth; or by any other criteria they generally use. The number of tests given on one day was usually two, although one or three samples were occasionally used. Only one type of product was tested on any day. For the report blank the nine-point rating scale of Peryam and Girardot (9) was adapted to staling with ratings from *very stale* to *very fresh*. A trained panel of 20 members, selected on the basis of ability to detect staling changes, was used throughout the study. To permit statistical analysis of the panel judgments the lowest rating, *very stale*, was given the value 1. Other ratings up the scale were given consecutive integers to 9 for *very fresh*.

*Compressibility.* The firmness values given in this report were obtained with the Baker Compressimeter (5). The cake was cut in a miter box and center sections of  $\frac{1}{2}$ -in. slices were used, generally  $\frac{1}{2}$  by 1 by 1 in.; when necessary the area was decreased in order to keep the measurements within the scale of the instrument. The compressimeter was operated to 2.5-mm. compression. Firmness readings are expressed in g. per sq. in.

*Crumbliness.* Crumbliness was determined by a variation of a procedure reported by Bice and Geddes (5). Eight 1-in. cubes were placed in a U.S. No. 4 sieve equipped with a catch pan and lid. After 10 minutes of shaking in a mechanical shaker, the cubes and sifted crumbs

were weighed and the percentage of crumbs calculated.

**Moisture.** To simulate home storage of a type to permit cakes to lose moisture rapidly, unwrapped cakes were kept in a metal drawer in a food laboratory. Other cakes were wrapped in heavy aluminum foil with the edges tightly folded. This effectively prevented moisture loss during the storage period. Moisture loss was found as the difference in weight of the cake when fresh and at the time of testing for freshness.

### Results and Discussion

Initial freshness tests were made by the panel 3 hours after the cakes were baked. Although the ratings varied somewhat, as shown in Tables I and III, there were no significant differences between values for white and yellow cakes, or between the conventional layers and those which were canned. In view of the instructions to the panel, sensory judgments included the effects of the changes in flavor, aroma, and physical properties which occurred during storage. No attempt was made to identify or measure the individual factors which entered into such judgments.

**Effect of Oxidation.** The importance of oxidative changes in the staling of white and yellow layer cakes is shown in Table I. Cakes stored in oxygen were consistently rated less fresh than those in nitrogen, except for white cakes after storage for 1 day. Mean squares from analysis of variance are given in Table II. The mean squares due to treatment were found to be significant at the 1% level for both types of cakes, which indicates that oxidative changes reduced the freshness of these products. A higher rate of staling in oxygen than in nitrogen was further established by partitioning the sum of squares of the treatment  $\times$  storage time interaction. For both white and yellow cake the mean squares associated with the treatment  $\times$  linear trend were significant at the 1% level and the deviations were negligible. Thus, it appears that oxygen caused a reduction of freshness which increased with the storage time.

It seems reasonable to assume that the panel found the cakes acceptable until the rating fell appreciably below 5 (neither fresh nor stale). On this basis oxidation of cakes became of major importance after storage for more than 19 days. At the 19th day all were rated 5 or above (the rating 4.94 for yellow cakes stored in oxygen is not appreciably below 5). While yellow cakes stored in nitrogen were rated approximately 5 (4.98) after 33 days, those stored in oxygen were slightly stale (4.22) after 27 days. Similarly, white cakes in nitrogen were rated 5.35 after 27 days while those in oxygen were rated only 4.42.

The difference in composition between the white and yellow cakes was the presence of the egg yolks in the latter. Since staling of both white and yellow cakes was more rapid in oxygen than in nitrogen, oxidative changes were of importance with both formulations and could not be explained by the oxidative changes of the egg yolks only. Further, since yellow and white cakes staled at similar rates in oxygen, it appears that the oxidative changes of the egg yolks did not appreciably reduce the shelf-life of baked cakes under conditions of these experiments.

Taste testing of canned cakes was discontinued when microbial

TABLE I  
EFFECT OF OXIDATION ON THE STALING OF YELLOW AND WHITE LAYER CAKES  
STORED AT 70°-72°F. (21°-22.5°C.)

STORAGE TIME	CAKES IN NITROGEN		CAKES IN OXYGEN	
	Panel Rating <sup>a</sup>	S.D.	Panel Rating <sup>a</sup>	S.D.
days				
			Yellow cakes	
0	8.22	0.90	8.22	1.03
1	7.80	1.45	6.95	1.63
4	7.15	1.53	6.40	1.43
12	6.60	1.69	5.70	1.42
19	5.06	1.92	4.94	1.88
27	5.21	1.94	4.22	1.76
33	4.98	1.87	3.45	1.86
			White cakes	
0	7.95	1.05	7.95	1.03
1	6.84	1.20	7.00	1.12
4	6.95	0.97	6.55	1.69
12	5.85	1.68	5.35	1.91
19	5.67	1.94	5.11	1.88
27	5.35	1.75	4.42	1.62

<sup>a</sup> Based on the rating scale: 9, very fresh; 8, fresh; 7, moderately fresh; 6, slightly fresh; 5, neither fresh nor stale; 4, slightly stale; 3, moderately stale; 2, stale; 1, very stale.

growth was detected in cans stored under nitrogen for 33 days. The contaminating organism produced rope, which was detected microscopically inside the cake. Bacteriological examination showed that it was a gram-positive rod which produced spores on nutrient agar and formed pellicles in trypticase soy broth. All these characteristics were indicative of *Bacillus mesentericus*. Dack (6) showed that special precautions must be taken with bread canned anaerobically, to ensure against growth of *Clostridium botulinum*. These observations have led to questions regarding the safety of canned bakery products, including cakes, unless special precautions in formulation have been taken to ensure against bacterial growth.

TABLE II

SOURCE OF VARIATION	YELLOW CAKE		WHITE CAKE	
	d.f.	m.s.	d.f.	m.s.
Total	279	4.4304	239	2.7945
Treatment <sup>a</sup>	1	60.5430**	1	9.8780**
Storage time	6	93.8179**	5	47.2698**
Linear trend	1	511.0034**	1	201.2563**
Deviations	5	10.3809**	4	8.7732**
Treatment $\times$ Storage	6	2.4645*	5	1.7623
T $\times$ Linear	1	9.3519**	1	7.7274**
T $\times$ Deviations	5	1.0871	4	0.3848
Judges	19	10.1963**	19	6.0237**
Judges $\times$ Trt.	19	1.1409	19	1.1761
Judges $\times$ Storage	114	2.4475**	95	2.0076**
Judges $\times$ Str. $\times$ Trt.	114	0.9073	95	0.8985

<sup>a</sup> Atmosphere in the can.

*Effect of Moisture Loss.* Storage temperatures were between 70° and 78°F. (21° and 25.5°C.). As shown in Table III, the panel readily detected loss of moisture and interpreted it as staling. After storage for 4 days both yellow and white layer cakes which were protected from moisture loss were judged *slightly fresh* (6.20 and 6.10 respectively). At the same time the unwrapped cakes were judged to have staled much more. The yellow cake was rated *stale* (2.10) and the white cake *slightly stale* (4.30).

#### The moisture loss from white and yellow cakes after equal storage

TABLE III  
EFFECT OF MOISTURE LOSS ON THE STALING OF YELLOW AND WHITE  
LAYER CAKES STORED AT 70°-78°F. (21°-25.5°C.)

Storage Time days	Cakes Unwrapped			Cakes Foil-Wrapped <sup>c</sup>	
	Panel Rating <sup>a</sup>	S.D.	Moisture Loss <sup>b</sup> %	Panel Rating <sup>a</sup>	S.D.
	Yellow cakes				
0	7.90	1.24	0.0	7.90	1.05
1	6.15	1.56	3.6	7.30	1.49
4	2.10	0.95	10.5	6.20	1.63
11	...	...	...	5.50	1.24
14	...	...	...	4.65	1.91
White cakes					
0	7.82	1.51	0.0	7.82	1.22
1	6.95	1.24	3.8	6.75	1.32
4	4.30	1.10	9.7	6.10	1.76
7	2.55	1.63	15.6	5.20	1.91
14	...	...	...	4.80	1.75

\* Same as for Table I.

<sup>b</sup> Loss in weight expressed as percent of original weight of cake. Original moisture content of yellow cake 23%; white cake, 29%.

\* Moisture loss negligible.

periods was approximately the same. It appears in Table III that equal loss of moisture resulted in more rapid staling of yellow than of white cake. However, the original moisture content of the white cake (29%) was considerably higher than that of the yellow cake (23%). Thus, in a general way the extent of staling due to moisture loss is related to the moisture content of the cake.

The cakes wrapped in aluminum foil maintained their original weight within 0.4 g. during the entire test. These cakes also staled, though more slowly (Table III). Studies were made of changes in firmness and crumbliness to determine whether staling might reasonably be related to changes in these physical properties.

*Staling vs. Changes in Physical Properties.* Both white and yellow cakes wrapped in metal foil (Table III) and the canned cakes stored under nitrogen (Table I) staled appreciably in the first few days, although they did not lose moisture and the latter were protected against oxidative changes. It might be expected that staling under these conditions was related to changes in crumbliness and firmness of the cakes. There is no objective means of measuring changes in texture, but it was observed that the cakes became harsh during storage.

TABLE IV  
CRUMBLINNESS OF YELLOW AND WHITE LAYER CAKES STORED AT 70°-72°F. (21°-22.5°C.)

STORAGE TIME	YELLOW CAKE		WHITE CAKE	
	Foil-Wrapped : Crumbs	Canned — in Nitrogen : Crumbs	Foil-wrapped : Crumbs	Canned — in Nitrogen : Crumbs
days	%	%	%	%
0	55	65	75	60
1	64	54	68	72
6	72		59	
12	62	74	48	55
19	53	55	— <sup>a</sup>	50
26	51	54	— <sup>a</sup>	48
33	— <sup>a</sup>	62	— <sup>a</sup>	50

\* Mold growth prevented further testing.

Crumbliness does not seem to be a factor simply related to cake staling (Table IV). Crumbliness of foil-wrapped yellow layers increased slowly for 6 days, then decreased somewhat. The crumbliness of canned yellow cakes followed a similar pattern but the period of increasing crumbliness was 12 days. Crumbliness of white cake decreased during storage for 19 days, then remained constant.

*Firming.* Firmness of both white and yellow cakes increased throughout a storage period up to 33 days (Table V); the greatest increase occurred during the first day. The rate of increase became pro-

TABLE V  
FIRMNESS OF YELLOW AND WHITE LAYER CAKES STORED AT 70°-72°F. (21°-22.5°C.)

STORAGE TIME	YELLOW CAKE		WHITE CAKE	
	Foil-Wrapped	Canned — in Nitrogen	Foil-Wrapped	Canned — in Nitrogen
days	g/sq in	g/sq in	g/sq in	g/sq in
0	29	29	31	36
1	37	43	35	41
4	44	43	52	
12	49	48	57	61
19	55	52	— <sup>a</sup>	58
26	55	56	— <sup>a</sup>	58
33	— <sup>a</sup>	57	— <sup>a</sup>	63

<sup>a</sup> Mold growth prevented further testing.

gressively less until the fourth day, after which it was approximately constant to the end of the experiment.

Approximately one-fourth of the total firming occurred on the first day, and one-half within the first 4 days. Changes in the panel judgment of freshness show a similar relationship. Taking the values for canned yellow cakes stored in nitrogen, since these were used for panel tests for the same length of time, it was found that one-sixth of the total staling occurred within one day and one-third within the first 4 days (Table I). Panel tests of white cakes stored in nitrogen were made for 27 days (Table I). Freshness values after storage for 1 and 4 days were approximately the same. They show that approximately 40% of the total staling occurred within 4 days. It thus appears that a relationship exists between increase in firmness and loss of freshness during the early period of storage.

#### Acknowledgments

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## RELATION OF THE RIGIDITY OF FLOUR, STARCH, AND GLUTEN GELS TO BREAD STALING<sup>1</sup>

J. E. CLUSKEY, N. W. TAYLOR, AND F. R. SENTI

### ABSTRACT

A penetrometer was modified to obtain load-depression curves of gels of wheat flour, starch, and gluten from which values of rigidity (Young's modulus) were calculated. Rigidity of flour and starch gels increased with time; this increase was most rapid within 1 to 2 days after preparation. Relatively less firming occurred for gluten in the same time. Reheating the flour and starch gels restored their original elasticity, whereas those of gluten were not affected by reheating. Results of this study indicate that firming of bread crumb during staling can be attributed mainly to the starch fraction.

Crumb compressibility measurements have been commonly used to follow the bread staling process (4,5). Much less information about starch gel compression has appeared, especially about wheat starch gels. Bechtel's (2) work on corn starch gels and their rigidity changes showed that dilute corn starch gels increase in rigidity with time. This effect has also been reported by Sterling (11) in his quantitative studies on confectionery jellies of thin-boiling corn starch in the presence of high sugar content.

That staling bread develops crystallinity and that the molecular order displayed is attributable entirely to the starch component of bread were shown by Katz (8) in his X-ray studies. Later, Hopper (7), using X-ray techniques to supplement compressibility studies, showed that retardation of compressibility changes in bread by monoglycerides qualitatively corresponded to retardation in rate of starch crystalliza-

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tion, thus linking crumb firming with starch crystallization. Hellman *et al.* (6), in their starch gel aging studies, presumed an intimate relation existing between crystallization processes and firming of bread. Bechtel<sup>2</sup> and Bechtel and Meisner (3) reported firming of dilute starch gels and related this to the firming of bread.

That the starch fraction of bread is the primary source of firming has been suspected for a long time, but to the authors' knowledge quantitative data have never been cited for starch gels with water contents approximating those in bread. The purpose of this paper is to present findings made on wheat starch, gluten, and flour gels at moisture contents comparable to those found in bread.

### Materials and Methods

Wheat starch was prepared by the batter process (1) from a commercial second clear flour, milled from hard red winter wheat. The gluten, from a commercial Ponca flour, was of two types: (a) crude gluten which was hand-washed from the flour under a stream of 0.1% sodium chloride solution and dried *in vacuo* over phosphorus pentachloride, and (b) precipitated gluten prepared as follows: The elastic gluten ball, as isolated above, was dissolved in 0.03M acetic acid in a Waring Blender. This acidic solution was centrifuged to separate the remaining starch. After decantation the acidic solution was heated to 98° to destroy enzyme activity, cooled, and neutralized to pH 6.6. The gluten precipitated at pH 5.9. It was then vacuum-desiccated over phosphorus pentachloride. Lusena's (9) baking tests have shown that gluten purified by acid extraction was substantially unaltered. Five straight flours from lots milled experimentally on a Buhler mill were used, namely: Ponca, Golden Ball, Willet, Lee, and Mindum.

A Precision penetrometer<sup>3</sup> was adapted for use as a compressimeter. In the present experiments the cone of the instrument was removed. An aluminum platen was made, 23 mm. in diameter with a depression in the center which received the tip of the penetrometer's plunger. This disk was placed on top of the gel plug. Weights were applied in increments up to 650 g. and the depression of the platen with each weight was observed after 1 minute. The weights were then removed in reverse order of their addition to observe the extent of irreversible flow. The compressimeter scale read directly in 0.1-mm. units and could be estimated to 0.01 mm.

A plot of decompression vs. load yielded a curvilinear line from

<sup>2</sup> Bechtel, W. G. Unpublished results.

<sup>3</sup> Manufactured by the Precision Scientific Company, Chicago, Ill., U.S.A. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

which a slope was calculated as described below. From this slope the Young's modulus, Y, or modulus of compressibility, was calculated.

$$Y = \text{deformation}/\text{unit length} \times \text{unit force}$$

Therefore,  $Y = \text{initial plug thickness}/\text{area of platen} \times \text{slope of the load-depression diagram}$ .

*Preparation of the Gel Plugs.* A dough was prepared by mixing a calculated amount of water and the raw wheat component in a small beaker. Stirring and kneading with a glass rod or a metal spatula followed, and was continued until, from all appearances, the sample was wetted uniformly. The water contents were near the range of interest in bread technology, 37.5–50.5% water (total weight basis). The paste was poured or packed into a brass mold, sealed, and heated.

Two C-shaped sections of brass, 18 mm. high, were used to form the cylindrical mold 23 mm. in diameter. This mold was placed on a square glass plate which was coated with light vacuum grease (silicone). Into the cylinder was fitted a 22-mm.-diameter microscope cover glass and the paste was poured or packed in. Another cover glass was added on top of the paste and the assembly was covered with a glass base plate. The filled mold was then secured with a clamp and placed in an oven. The heating time required to reach 100°C. was controlled by a variable resistor. Average heating times for the plugs were: wheat flour, 200 minutes; wheat gluten, 175 minutes; and wheat starch, 100 minutes. No effect of heating time was anticipated.

After the oven reached 100°C., the assembly was removed, cooled rapidly to room temperature on an ice pack, and the gel removed from the mold. The cover glasses were not removed but were left as protective base plates to keep the bottom and top surfaces flat. The cooled gel plugs were placed immediately under light mineral oil contained in brass or steel test cups. The plugs were not removed from under the oil during the whole series of tests as this minimized moisture losses.

In reheating experiments, the oil-covered plugs were placed in an oven at 100°C. for 0.5 hour, removed, and cooled to room temperature. The testing cycle was then repeated.

### Results and Discussion

Preliminary experiments showed that little moisture loss or redistribution occurred in the samples during the baking procedure. A starch gel plug made from a starch-water slurry containing 50.5% water (total weight basis) was sectioned into three disk-shaped parts and the moisture of each part determined by vacuum-oven drying at

105°C. The results were : Top 50.5%, mid 49.2%, base 50.6% water. These analyses show that only a small amount of water was lost in the baking process. The lowered water content observed in the mid-section indicates some migration of water toward the outside of the plug. Such a migration would be the result of early gelatinization and water absorption by the starch at the hotter surface of the plug during baking, but the close agreement in analyses of the three sections indicates that no large amount of such nonuniform gelatinization occurred.

No substantial amount of moisture was lost either in aging or in heating the plugs under oil. Experiments showed that approximately 0.1% moisture was lost in the aging of a 50.5% gel over a period of 11 days. When a plug was reheated after aging and compression testing, a loss in weight of 0.5–0.7% occurred.

*Wheat Starch Gels.* Load-depression curves are given in Fig. 1 for a starch gel which contained 50.5% water by weight. It can be seen that the plots, obtained after aging for the indicated times, are somewhat curved, and also show some mechanical hysteresis or creep, since the depressions are different on addition and reduction of the load.

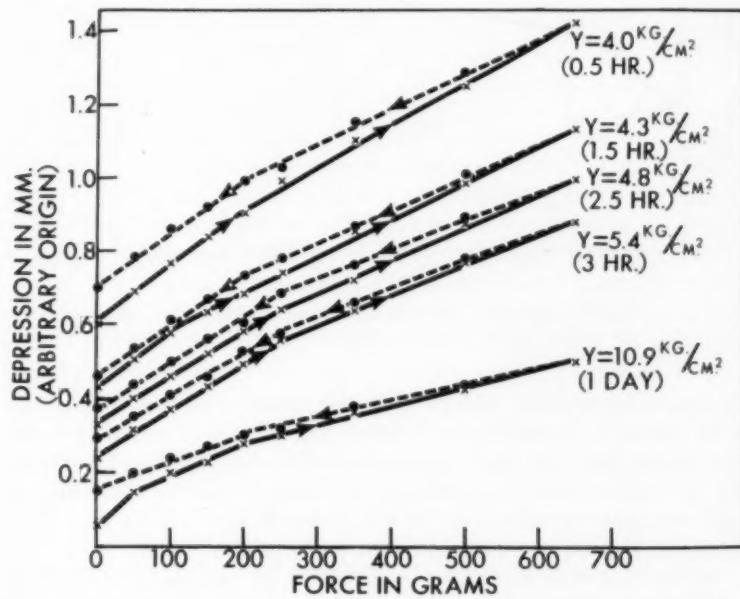


Fig. 1. Load-depression curves for a wheat starch gel containing 50.5% water by weight.

However, the parts of the curves obtained at loads greater than 200 g. are reasonably straight and thus can be used to obtain Young's modulus. The parts of the curves observed from 0 to 200 g. of load were relatively irregular and less reproducible. This curvature could be the result of poor contact of the plug ends with the bottom of the cup or the aluminum platen, or could be caused by inhomogeneities in the plug or by the character of the gels themselves.

Firming of the starch gels is shown in Fig. 1 by the decreasing slopes with consequent increase in modulus upon aging. After 1 day the modulus is greater than twice the initial value.

The effects of further aging are seen in data from five 50.5% wheat starch gel plugs which are shown in Fig. 2, as Young's moduli plotted

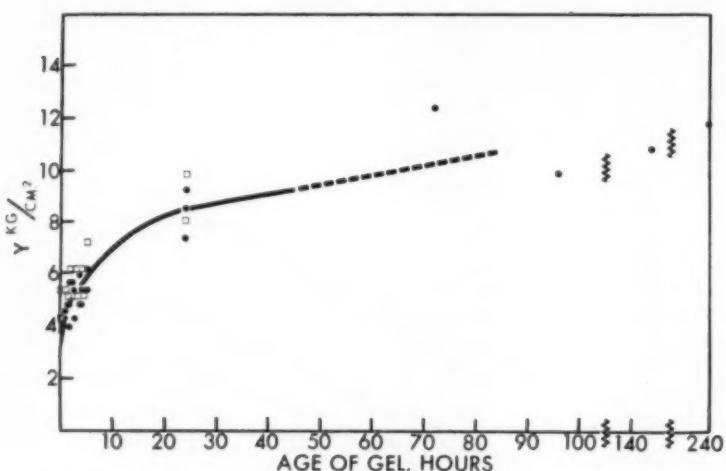


Fig. 2. Effect of aging on Young's modulus values of wheat starch gel containing 50.5% water by weight. Circles represent points taken before any reheating. Squares show points after reheating.

against hours of aging after heating. It may be noted that during the first 24 hours a rapid change occurs. After 24 hours relatively little change occurs in the curve. Data represented by circles on the curve are those taken before any reheating of the plug was done. The squares represent data obtained after the plug was reheated and indicate that reheating reduces the modulus to near the original value.

Effect of moisture content in the starch gels on their mechanical properties was also investigated. Results on wheat starch gel plugs with water contents different from 50.5% are shown in Table I. In-

TABLE I  
WHEAT STARCH GELS

WATER % (wet basis)	AGE OF GEL		MODULUS Y kg/cm <sup>2</sup>
	hours	days	
48.4	0.5	1	4.0
		4	5.0*
		5	22
		6	6*
		8	9
		1	11
		2	15
45.6	1.5	1	10
		2.5	10
		3.5	10
		1	19
		2	9*
			16
42.4	0.5	1	8
		1.0	10
		2.5	10
		3	20
		4	10*
38.7	1.0	1	7
		1.5	9
		1	15
		5	16
			9*

\* Modulus value obtained after the aged gel was reheated under oil at 100°C. for 0.5 hour.

spection of the table reveals that these gels also increase in rigidity with time and that the process is essentially complete in 1 day. Furthermore, reheating reduces the modulus to near its original fresh value. Thus, these features appear to be general in starch gels with water contents near those used in bread baking.

The starch gels containing 45.6% water or less appeared to be indistinguishable in their moduli, both fresh and aged. However, with larger quantities of water the initial or fresh moduli were much lower. Hellman, Fairchild, and Senti (6) have shown that there is a critical moisture content necessary before a given heat treatment will cause loss of original starch granule crystallinity. These workers demonstrated that as a result of heat treatment starch systems containing more than 43% water lost all type A crystallinity demonstrable by X-ray diffraction. In the present study it is shown that with a moisture content higher than about 45% the initial modulus is one-half as great as in gels with lower water content. Although the data are not extensive in this so-called "critical" range, correlations between water content, firmness, and crystallinity appear to be possible.

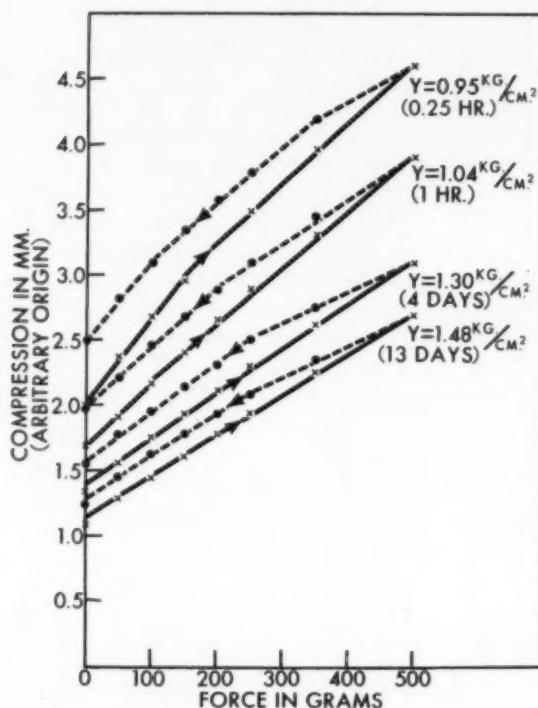


Fig. 3. Load-depression curves for a wheat gluten gel containing 39.3% water by weight.

*Wheat Gluten Gels.* The load-depression curves for a gluten gel plug containing 39.3% water are shown in Fig. 3. These curves are qualitatively similar to the starch gel curves, but the decrease in slope or increase in modulus is progressive through the 13 days of observation. Further, the increase in modulus is much smaller as a percentage change than in the starch gels.

Data from three wheat gluten gel plugs of different water content appear in Table II. Plugs I and III were prepared from crude Ponca gluten. Plug II was made with precipitated gluten. The procedure of reheating aged gels was followed, and a marked difference from the starch and flour gels (see below) was demonstrated. This step had no effect on the pure gluten plug. The modulus continued to rise, that is, staling was not retarded in plug II. In plugs I and III the arresting of modulus increase or slight rise in modulus can be attributed to residual

TABLE II  
WHEAT GLUTEN GELS

PLUG	MOISTURE IN GEL	AGE OF GEL		MODULUS Y $\text{kg/cm}^2$
		%	hours	
I	39.3	0.25		0.95
		+1.0		1.04
			4	1.3
			5	1.4
			7	1.4
			13	1.5
			14	1.5*
		+2.0		1.8
		+4.0		2.0
			25	2.7
II	37.3	0.5		1.2
		1.5		1.4
		2.5		1.4
			1	1.4 (19.4%) <sup>b</sup>
		+1.0		1.6*
			2	1.6
III	36.6	0.5		1.5
		2.5		1.7
		3.5		1.9
			1	2.1 (40%) <sup>b</sup>
			2	2.4
		+2.0		1.9*
				2.7
			1½ months	3.3

<sup>a</sup> Value of modulus after reheating in 100°C. oven for 0.5 hour.

<sup>b</sup> Percentage increase in modulus over the initial after 1 day aging.

starch in the gluten after hand-washing. Sullivan (12) has stated that the gluten ball, after separation by hand-washing, may contain some 6% starch.

A comparison of modulus increase after 1-day aging is given in Table II for gluten plugs II and III. There was a 19.4% increase for plug II, whereas in plug III a 40% increase is shown. Wheat starch gels in this moisture range increase in firmness between 90 and 100%.

Gluten plug III was tested after 1.5 months and its modulus had increased twofold from the initial value. In starch and flour plugs the maximum firmness was reached in about 1 day.

*Wheat Flour Gels.* Figure 4 shows a plot of the Ponca wheat flour at 46.1% moisture. Load-depression plots of the flour resemble the starch plots but, in general, the ascending curves are more nearly linear, especially at the higher moisture concentration. The reduction of slope with aging can be observed. Other investigators (5) have shown that bread crumb also gives nearly linear curves under appropriate loads.

Compressibility measurements were carried out on several varieties of wheat flour shown in Table III. Three values are given from each experiment: the initial, 1-day-old (with percentage modulus increase), and "reheated" values. When the flours were reheated, compressibility returned nearly to initial values.

The last five samples in Table III are different flours, all of 37.5% moisture content. Pastes suitable for gelling could be made from flour at this low-moisture level, whereas with wheat starch alone this was not possible. Evidently other components of the whole flour aid in moisture distribution. The lesser percent of modulus increase for the Willet flour after 1 day is repeatable and somewhat anomalous. Perhaps this behavior can be attributed to the relatively higher gluten content of Willet flour (about 17.4%) and/or to wheat quality.

Since gluten increases in rigidity relatively slowly, a higher gluten content should result in a lesser increase in modulus of the flour. Because of the small number of samples of each variety included in

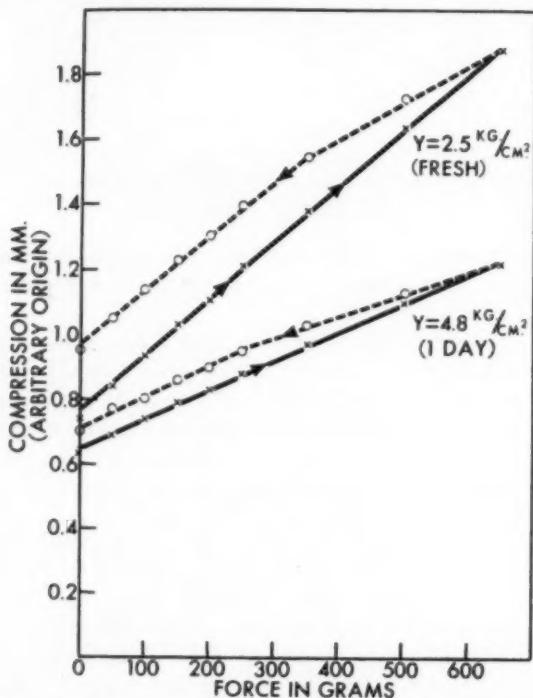


Fig. 4. Load-depression curves for a Ponca wheat flour gel at 46.1% moisture.

the study, it was not possible to draw conclusions regarding the effect of the original wheat protein content upon the compression moduli of the plugs; however, rheological behavior of doughs is no doubt dependent on the physical properties of the gluten.

*Comparison with Staling of Bread.* Results reported in the previous sections demonstrate that starch gels reproduce qualitatively the staling phenomena in bread, not only in their firming with age, but also in their refreshening behavior on reheating. In contrast, gluten gels showed only relatively slight firming with age and showed no refreshening on reheating. These observations substantiate the general belief (10) that the firming of bread is due mainly to the starch component. Still, there appears to be a minor effect of the gluten component in increasing rigidity which was previously unanticipated.

If the firming curve of starch in Fig. 2 is compared with the load-compression results of a typical bread crumb (5), it appears that the starch firmness curve becomes flatter after 2 or 3 days' aging than does the curve for bread crumb. This difference might be due to a higher water content in the starch gels under consideration than in bread crumb. The water content of a gel might well be expected to influence

TABLE III  
WHEAT FLOUR GELS

FLOUR VARIETY	WATER %	SLOPE OF CURVE	CONDITION OF GEL	MODULUS kg/cm <sup>2</sup>	INCREASE %	
					%	
Ponca	46.1	0.018	Fresh	2.5		
		0.009	1 day old	4.8		
		0.016	Reheated	2.7		
Red Chief	47.2	0.022	Fresh	2.1		
		0.011	1 day old	4.1		
		0.017	Reheated	2.6		
Willet	39.4	0.013	Fresh	3.4		
		0.005	1 day old	9.5		
		0.011	Reheated	4.1		
Willet	37.5	0.008	Fresh	5.6		
		0.005	1 day old	8.9		
		0.008	Reheated	5.6		
Red Chief	37.5	0.013	Fresh	3.4		
		0.005	1 day old	9.9		
		0.012	Reheated	3.7		
Mindum (durum)	37.5	0.011	Fresh	4.1		
		0.004	1 day old	11.1		
		0.010	Reheated	4.5		
Golden Ball (durum)	37.5	0.013	Fresh	3.7		
		0.005	1 day old	8.9		
		0.011	Reheated	4.1		
Lee	37.5	0.008	Fresh	5.6		
		0.004	1 day old	11.1		
		0.007	Reheated	6.4		

the rate as well as extent of retrogradation or firming. On the other hand, the difference in the firming curves may reflect mechanisms in the bread staling process other than firming of the starch fraction. The continued gradual firming of the gluten fraction, as shown in Table II, may contribute significantly to the firming of bread after several days' aging.

A quantitative comparison of these results with rigidity moduli in bread cannot be easily made because of the complex structure in bread. First, the flour is a complex inhomogeneous mixture, and second, this mixture is formed in bread into an elaborate cellular network. At least, it can be noted that the percentage increase in the "firmness" or Young's modulus of the starch and flour gels appears to be sufficient to account for most of the firmness increase in bread on aging.

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# A STUDY OF THE EFFECT OF DRYING CONDITIONS ON THE SUITABILITY FOR STARCH PRODUCTION OF CORN ARTIFICIALLY DRIED AFTER SHELLING<sup>1</sup>

MAJEL M. MACMASTERS<sup>2</sup>, M. D. FINKNER<sup>3</sup>, MARGARET M. HOLZAPFEL<sup>2</sup>,  
J. H. RAMSER<sup>4</sup>, AND G. H. DUNGAN<sup>4</sup>

## ABSTRACT

Corn from five crop years (1952-1956) was harvested at approximately 30 and 20% moisture content, and dried at six air temperatures ranging between 120°F. (48.9°C.) and 200°F. (93.3°C.), each with air at 15% relative humidity (R.H.) and 40% R.H., respectively. Drying conditions were such that the corn doubtless reached the temperature of the drying air. Controls were dried in ambient air.

On the basis of recovery and quality of the starch, acceptable processing of corn was obtained in the laboratory when the grain had reached a temperature as high as 160°F. (71.1°C.) during drying. The initial moisture content of the corn and the prevailing conditions of relative humidity were relatively unimportant with respect to these two processing characteristics. However, since corn oil is an important by-product, commercial processing requires the separation of a high yield of high-quality oil. Corn with dead germ is difficult to process, and the subsequent yield and quality of the oil are low. Hence, the 2,3,5-triphenyltetrazolium chloride color test for viability was used to determine the state of the germ. Corn that had reached temperatures above 140°F. (60°C.) during drying showed a definite decrease in viability and was considered to be lowered in quality for use in starch production.

Corn wet-millers are seriously concerned about the increasing volume of artificially dried corn coming into the market. Farmers and elevator operators who are drying corn often consider only its feeding value, because corn is so generally considered as a feed grain. Yet in 1954, for example, over 25% of all corn sold off the farm was used industrially. Of this, more than half was processed by wet-millers (6).

Improper drying affects the protein and starch, and thus creates numerous processing problems: (a) grinding is difficult and incomplete, with consequent loss of starch into byproduct feeds; and (b) separation of starch and protein at the centrifuges is poor, with resulting low recovery of starch and poor quality of the recovered starch. Some other general complaints are: (a) difficulty in drying the corn gluten fraction; (b) poor germ separation; (c) low yield of oil from

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<sup>2</sup> Northern Utilization Research and Development Division, Peoria, Illinois, one of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture.

<sup>3</sup> Biometrical Services, ARS, USDA. Present address: New Mexico College of Agriculture and Mechanic Arts, State College, New Mexico.

<sup>4</sup> Illinois Agricultural Experiment Station, Urbana, Illinois.

germ; and (d) poor color and high fatty-acid content of the oil. In fact, the processing difficulties are so great that some corn wet-millers refuse to purchase corn known or suspected to have been artificially dried. Other industrial users of corn encounter comparable troubles when the grain has been carelessly dried.

The work reported here was part of a study undertaken to determine artificial drying conditions which might be used to obtain corn that is still acceptable as raw material for industrial production of starch by wet-process milling. Previous work was carried out on dried ear corn (3, 4); increasing use of the picker-sheller, or the corn combine, led to the current investigation on drying of shelled corn.

### Materials and Methods

**Materials.** Corn was grown, harvested at approximately 30 and 20% moisture content, shelled (or harvested by picker-sheller), and dried at the Illinois Agricultural Experiment Station in 1952, 1953, 1954, 1955, and 1956. Illinois 1277 hybrid corn (WF9 × M14) (187-2 × I 205) was used throughout, except that all samples harvested at the higher moisture content in 1956 were Illinois 805 hybrid (WF9 × 38-11) (C103 × Oh45). Corn that could not be dried at once was placed immediately in a constant-temperature room at 25°F. (-3.9°C.) and held there until the drier was available.

**Drying.** The drying method was that previously described (3), except that a finer-mesh screen was used to support the shelled corn in the drier. Corn was dried from approximately 20 and 30% moisture content at air temperatures of 120°, 130°, 140°, 150°, 160°, 180°, and 200°F. (48.9°, 54.6°, 60°, 65.6°, 71.1°, 82.2°, and 93.3°C.), with air at 15% R.H. and 40% R.H. Since the drying time always amounted to at least 1 hour, the corn doubtless reached the temperature of the drying air. One lot of corn at each initial moisture content was dried at each temperature with air at each relative humidity. A control was air-dried at ambient temperature and R.H. from each initial moisture level.

**Processing.** The laboratory processing method used was described previously (4).

**Viability.** Viability was determined by use of 2,3,5-triphenyltetrazolium chloride (1,5).

**Analytical Methods.** The analytical methods were those previously described by MacMasters *et al.* (4).

**Analysis of Data.** The data were analyzed statistically by use of the analysis of variance, and the various treatment effects were tested for significant differences by applying Duncan's multiple range test (2).

### Results and Discussion

Complete processing data are presented in Table I.

Processing data which were subjected to the analysis of variance included percentage starch recovery and percentage of protein in starch. These analyses are presented in Tables II and III. The mean percentage of starch recovery of the controls was significantly greater than the mean for the samples dried under different temperatures and relative humidity only in 1952; however, there was a consistent though nonsignificant trend in favor of the controls for the other four years in which the test was run. Differences in starch recovery between the high and low initial moisture content of the controls was small, and could be explained on the basis of random variability.

The effect of drying temperatures on starch recovery was evident in four of the five years under test. Only in 1952 did temperature differences fail to attain significance, and in that year the treatment at 200°F. (93.3°C.) was not included in the test. Initial moisture of the corn influenced starch recovery in two of five years with the low moisture content contributing to higher starch recovery. Relative humidity effects were significant in 1955 only.

Differences with respect to percentage of protein in starch between the mean of the controls and the mean of the artificially dried samples were nonsignificant in all years. Likewise, differences between initial moisture of the controls could be explained on the basis of random variability. Temperature differences were significant in 1953 only, although the higher temperatures consistently resulted in higher amounts of protein in starch in all other years. Moisture differences were significant in four of five years; however, no consistent effect could be observed with respect to initial moisture. In 1952 and 1954, high initial moisture resulted in high percentage of protein in starch, while in 1953 and 1956 the reverse was observed. The protein in starch was influenced by relative humidity in 1952, but no significant effects were observed in the remaining years.

The combined analyses of variance for all years except 1952 are presented in Table IV. The data from 1952 were excluded because the drying treatment at 200°F. (93.3°C.) was not used that year. The combined analyses provided information on the variability among years and on the presence or absence of important interactions. It also allowed for broader interpretation regarding the main effects and provided more precise estimates of experimental errors.

Variability among years was significant in both the starch recovery and the protein in starch analysis. Wet-process corn millers are well

TABLE I  
EFFECTS OF DRYING CONDITIONS ON VIABILITY AND LABORATORY PROCESSING OF CORN FOR STARCH

	Drying Conditions					Viability by TTC Test	Starch Recover- y %	Processing Data (Moisture-Free Basis)					
	Initial Mois- ture %	Temp- erature of Air °F	R.H. of Air %	Time hours	Final Mois- ture %			Starch					
								in Starch	in Glucom-	Protein in Starch	Starch in Fiber		
Dried from approximately 30% moisture content													
1952	31.7	200	40	1.5	11.4	0	53.2	1.06	58.7	29.5	59.0	14.0	
	32.0	200	15	1.5	11.2	0	50.5	1.00	58.0	30.3	61.2	13.2	
	31.7	180	40	2.5	11.3	0	66.4	0.81	44.2	41.6	51.4	14.0	
	32.0	180	15	2.5	11.8	0	67.4	0.88	43.5	44.5	52.2	13.0	
	32.0	160	40	2.66	11.6	0	67.1	0.62	42.4	44.2	47.5	13.6	
	31.2	160	15	2.5	12.1	0	70.7	0.81	34.5	51.4	63.1	12.8	
	31.8	150	40	3.5	12.5	0	69.6	0.56	49.0	39.4	46.6	13.3	
	31.0	150	15	3.0	12.2	0	69.1	0.81	44.3	43.2	51.4	12.3	
	31.8	140	40	4.0	12.7	3	72.8	0.75	42.1	46.6	46.6	13.0	
	31.5	140	15	4.17	12.2	21	70.6	0.75	49.4	35.5	40.1	12.5	
	32.0	130	40	6.0	12.1	32	72.5	0.56	41.9	45.8	44.5	12.8	
	31.2	130	15	5.5	11.8	50	72.6	0.69	42.1	45.6	40.3	12.5	
	31.8	120	40	8.25	11.7	75	72.0	0.81	43.9	44.3	45.4	13.1	
	31.7	120	15	7.5	10.0	69	66.2	0.69	52.2	36.6	49.8	12.5	
	32.0	Ambient air	(24 days)	7.4	89	76.3	0.75	35.6	51.0	43.1	12.6		
1953	28.2	200	40	1.25	11.7	0	62.1	1.00	44.7	39.3	53.6	13.6	
	28.0	200	15	1.50	12.0	0	69.1	0.96	38.2	44.1	51.3	13.5	
	29.4	180	40	2.75	11.6	0	58.4	1.44	34.2	47.1	49.5	15.1	
	28.5	180	15	1.75	12.0	0	74.7	0.84	38.3	45.7	44.7	13.2	
	28.0	160	40	3.50	11.5	29	72.0	0.69	38.0	42.2	46.0	13.1	
	29.0	160	15	3.00	11.4	0	74.2	0.75	36.5	45.7	44.4	12.4	
	28.0	150	40	6.0	12.4	12	76.8	1.03	36.0	47.4	43.8	11.9	
	28.6	150	15	4.0	11.6	13	75.8	0.89	39.6	47.6	43.9	13.1	

(Continued on next page)

TABLE I (Continued)

Initial Mois- ture %	Temperature of Air °F	R.H. of Air %	Time hours	Final Mois- ture %	Processing Data (Moisture-Free Basis)						
					VIAILITY BY TTC TEST	Starch Recover- y	Protein in Starch	Starch in Gluten	Protein in Gluten	Starch in Fiber	Protein in Fiber
<b>1953 (cont.)</b>											
28.4	140	40	5.5	12.0	29	76.0	0.84	36.1	47.5	44.5	12.0
28.4	140	15	4.25	12.6	14	76.0	0.71	36.7	46.9	43.2	11.9
28.6	130	40	7.0	12.0	53	75.6	0.78	34.7	47.4	43.4	11.7
29.0	130	15	6.25	11.4	26	76.7	0.65	39.1	45.8	41.0	12.4
29.0	120	40	9.5	12.1	24	73.8	0.69	39.0	44.9	44.6	12.9
28.4	120	15	7.5	11.6	65	78.6	0.72	29.2	52.7	41.6	12.2
29.0	Ambient air	12.0	84	78.2	0.75	37.8	46.6	41.8	11.9		
<b>1954</b>											
29.8	200	40	1.3	12.5	0	78.9	1.19	36.0	43.3	40.2	19.8
30.0	200	15	1.1	12.8	0	76.3	1.06	36.9	42.2	40.1	15.0
30.0	180	40	2.2	11.5	0	84.5	0.84	28.4	51.4	37.4	15.6
30.0	180	15	1.5	13.0	0	81.6	1.25	43.2	43.3	35.3	14.8
30.0	160	40	3.8	12.0	0	84.4	0.88	33.4	45.8	32.4	14.3
31.2	160	15	2.5	11.6	0	84.6	0.69	32.8	47.1	32.5	14.4
30.0	150	40	4.8	11.6	8	85.6	0.69	30.9	50.4	33.2	13.6
31.8	150	15	3.5	11.0	0	82.8	0.94	34.0	45.6	32.2	13.9
30.4	140	40	4.25	11.6	28	84.3	0.86	29.6	49.8	33.0	13.4
30.8	140	15	2.6	12.3	20	84.4	0.75	34.8	44.4	30.3	13.4
29.6	130	40	5.1	13.1	10	85.6	0.79	30.4	48.0	31.2	13.3
30.5	130	15	3.85	13.0	40	84.3	0.81	28.6	49.8	31.2	13.1
30.0	120	40	7.5	12.0	28	79.4	0.75	36.4	44.5	31.4	13.4
31.2	120	15	6.15	11.6	45	81.1	0.94	33.5	45.8	35.4	13.4
31.0	Ambient air	11.0	56	86.4	0.82	29.9	48.0	31.6	13.1		
<b>1955</b>											
29.6	200	40	1.7	12.4	0	77.8	1.38	34.8	42.2	49.3	14.8
27.2	200	15	1.3	11.6	0	78.0	1.25	40.5	38.6	36.2	13.6
26.4	180	40	1.75	13.0	0	87.2	0.81	30.5	45.9	36.1	14.8
26.6	180	15	1.5	13.0	0	125	125	46.4	33.0	27.6	13.9

(Continued on next page)

## DRYING CONDITIONS VS. STARCH RECOVERY

TABLE I (Continued)

Initial Mois- ture %	Temperature of Air	Drying Conditions			VIALABILITY BY TTC TEST			Processing Data (Moisture Free Basis)		
		% P	R.H. of Air	Time hours	Final Mois- ture %	%	%	%	%	%
1955 (cont.)										
26.6	160	40	3.0	12.6	0	89.3	0.94	29.8	46.1	33.6
26.8	160	15	9.25	12.2	0	81.2	1.00	35.8	44.8	45.8
27.4	150	40	4.1	11.4	10	89.7	0.94	25.1	49.0	32.4
27.6	150	15	9.5	12.2	30	88.0	1.19	31.0	48.4	31.0
26.6	140	40	3.3	12.7	30	88.0	0.94	31.2	46.0	32.4
27.8	140	15	4.0	12.0	59	80.2	0.70	30.2	47.9	46.3
26.8	130	40	5.5	11.6	60	90.4	0.75	27.6	46.8	31.3
27.2	130	15	5.0	11.9	55	81.7	0.70	35.6	44.3	43.5
26.8	120	40	6.5	12.2	70	84.1	0.94	29.2	48.8	41.6
27.5	120	15	7.6	11.5	78	86.4	0.81	32.0	47.2	36.6
27.4	Ambient air	(7 days)	12.0	80	92.0	1.25	24.6	50.8	35.0	12.1
1956										
28.0	200	40	1.3	11.4	0	68.0	1.13	42.9	58.8	53.2
27.8	200	15	1.5	12.0	0	72.7	0.90	41.0	41.9	50.8
28.6	180 *	40	2.0	11.4	0	76.5	1.04	35.1	46.3	46.5
27.4	180	15	1.3	12.2	0	77.8	0.85	38.8	43.9	43.7
28.0	160	40	2.6	12.8	0	77.8	0.87	40.1	41.9	48.2
27.4	160	15	2.5	12.6	0	76.8	0.75	41.8	40.7	43.8
28.6	150	40	3.16	11.7	3	79.9	0.89	35.3	45.8	46.0
28.0	150	15	2.5	11.9	3	81.4	0.55	37.1	43.9	36.7
28.6	140	40	3.9	12.2	30	81.2	0.58	35.3	44.7	34.5
28.6	140	15	3.9	11.4	20	74.7	0.71	40.8	41.4	49.4
28.0	130	40	4.13	12.0	62	82.3	0.66	34.0	46.1	41.0
27.8	130	15	3.75	12.0	30	81.8	0.76	35.8	40.4	35.8
27.0	120	40	4.8	12.8	50	83.2	0.92	36.2	45.2	35.8
29.7	120	15	7.2	11.0	65	83.0	0.73	38.4	40.8	32.4
27.6	Ambient air		12.8	52	83.0	0.72	36.0	45.4	36.3	11.2

(Continued on next page)

TABLE I (Continued)

Drying Conditions						Processing Data (Moisture-Free Basis)						
#	Initial Mol- ture %	Temperature of Air °F	R.H. %	Time hours	Final Mois- ture %	Viability by TTC Test	Starch Recover- y %	Protein in Starch	Starch in Gluten	Protein in Gluten	Starch in Fiber	Protein in Fiber
Dried from approximately 20% moisture content												
1952												
21.0	180	40	1.5	11.8	0	69.9	0.87	40.4	47.9	49.8	12.9	
20.8	180	15	1.17	12.2	0	70.0	0.81	45.3	43.1	48.5	13.4	
20.8	160	40	2.33	12.0	2	71.6	0.88	36.4	51.0	48.4	12.7	
20.2	160	15	2.0	12.2	25	68.6	0.81	39.3	47.2	52.3	12.5	
20.6	150	40	2.75	12.0	66	72.0	0.61	46.9	41.9	44.8	12.6	
20.6	150	15	2.0	12.2	75	72.6	0.94	39.2	49.0	47.0	12.5	
21.0	140	40	3.5	12.2	93	71.6	0.75	43.0	53.0	46.7	12.8	
20.6	140	15	3.0	11.6	93	72.5	0.81	38.5	48.8	48.1	12.6	
21.0	130	40	4.75	11.6	98	75.2	0.81	33.1	52.9	46.2	12.8	
22.7	130	15	3.0	12.8	80	67.4	0.81	40.8	46.1	47.2	12.5	
20.8	120	40	6.1	11.6	98	75.3	0.75	36.7	49.1	45.2	12.7	
22.7	120	15	7.25	11.2	90	70.4	1.00	43.5	43.6	48.7	12.4	
21.4	Ambient air	(5 days)		8.2	99	76.3	0.75	43.6	44.3	43.7		
1953												
20.0	200	40	1.0	11.2	0	65.2	0.81	50.0	37.5	53.6	15.4	
22.4	200	15	1.25	11.0	0	69.6	0.81	43.0	41.1	54.5	12.8	
20.0	40	1.5	1.5	11.2	0	75.0	0.75	36.1	46.1	47.1	12.7	
20.0	180	15	1.0	12.6	0	77.8	1.19	37.3	46.0	39.6	12.9	
19.2	160	40	2.25	11.8	7	76.2	0.64	38.8	45.3	43.5	13.0	
22.0	160	15	2.25	11.8	9	74.1	0.62	38.4	46.3	43.2	12.5	
20.0	150	40	3.6	11.0	96	76.5	0.62	38.1	47.0	44.6	12.2	
20.0	150	15	2.0	11.4	32	75.9	0.88	37.4	47.3	46.0	12.4	
19.4	140	40	3.5	12.4	93	76.8	0.75	36.6	47.7	43.9	13.5	
19.6	140	15	3.0	11.0	94	75.3	0.58	42.4	44.0	42.2	12.4	
19.8	150	40	5.1	11.4	95	75.8	0.61	38.9	46.8	43.4	13.8	

(Continued on next page)

TABLE I (Continued)

Initial Mois- ture %	Temp- erature °F	R.H. of Air	Time hours	Final Mois- ture %	VIABILITY BY TTC TEST	PROCESSING DATA (Moisture-Free Basis)					
						Starch Recover- y	Protein in Starch	Starch in Gluten	Protein in Gluten	Starch in Fiber	Protein in Fiber
1953 (cont.)	130	15	3.1	12.0	98	75.8	0.52	38.6	44.6	43.4	13.1
	19.0	40	4.25	12.8	97	78.6	0.83	36.7	49.1	42.4	13.3
	120	15	3.75	12.2	99	77.5	0.61	36.6	48.2	42.9	12.2
	18.8	Ambient air	...	12.0	99	76.0	0.75	37.3	45.2	41.4	12.4
1954	200	40	1.0	12.5	0	78.2	1.44	35.2	45.1	42.5	16.1
	200	15	1.0	11.5	0	76.4	1.12	35.3	43.9	35.6	14.8
	180	40	...	12.0	0	81.7	1.06	28.3	52.0	43.6	15.8
	180	15	1.4	12.0	0	85.9	0.97	29.8	50.0	31.1	14.4
1955	160	40	2.3	12.0	20	84.2	0.86	27.8	51.0	32.6	13.6
	160	15	2.3	11.5	21	87.8	0.80	31.6	48.2	30.8	14.1
	150	40	3.5	11.4	21	87.8	1.21	26.8	51.0	31.6	14.0
	150	15	2.5	11.7	0	87.2	1.00	25.3	52.8	28.9	13.5
1956	150	40	4.1	11.8	22	87.8	0.93	30.7	50.4	32.0	14.0
	140	40	4.1	12.0	35	86.4	0.93	30.9	49.6	33.1	14.1
	140	15	2.8	12.0	72	85.0	1.01	31.3	53.7	33.2	13.4
	130	40	4.2	12.2	65	87.6	0.95	31.3	50.7	31.3	14.0
1957	130	15	4.8	11.3	95	85.6	0.80	30.0	56.0	32.1	13.8
	120	40	5.4	12.3	95	87.3	1.45	23.1	56.0	31.3	14.2
	120	15	6.8	11.8	68	84.5	0.84	36.4	46.6	31.3	14.2
	110	Ambient air	...	10.0	92	89.1	0.88	28.9	47.6	35.5	13.5
1958	200	40	1.5	12.0	0	85.2	0.88	29.9	47.6	41.4	15.8
	200	15	1.0	11.2	0	82.6	1.19	33.5	44.7	43.6	13.5
	180	40	...	11.5	0	89.1	0.88	28.9	47.6	35.5	13.5
	180	15	1.5	11.8	0	89.1	0.88	28.9	47.6	35.5	13.5

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TABLE I (Concluded)

Initial Mois- ture %	Temperature of Air °F	R.H. of Air	Time hours	Final Mois- ture %	VIABILITY BY TTC TEST	PROCESSING DATA (Moisture Free Basis)					
						Starch Recover- y	Protein in Starch	Starch in Gluten	Protein in Gluten	Starch in Fiber	Protein in Fiber
<b>1955 (cont.)</b>											
21.0	160	40	2.0	12.1	0	87.6	0.70	36.0	44.4	36.0	13.3
20.8	160	15	1.7	11.8	5	91.0	1.06	24.0	50.8	33.8	13.8
21.0	150	40	2.5	11.8	15	90.4	0.75	28.0	45.4	35.5	13.4
21.0	150	15	2.0	12.6	70	86.8	0.70	29.4	48.5	34.8	12.4
20.8	140	40	3.0	11.9	85	90.6	0.70	25.7	49.1	30.8	13.5
21.0	140	15	3.0	11.8	85	87.2	1.44	25.6	48.8	32.0	13.1
21.0	130	40	4.5	11.8	88	88.1	0.75	30.7	46.8	33.6	13.0
21.0	130	15	3.5	12.0	85	92.0	0.75	27.8	48.4	34.6	12.4
21.0	120	40	6.0	11.7	75	88.8	0.66	27.4	49.9	33.3	13.2
21.0	120	15	4.5	12.1	90	91.2	0.75	24.5	51.8	32.8	13.5
31.1	Ambient air	(7 days)		11.2	100	85.5	0.81	27.5	48.9	30.1	13.5
<b>1956</b>											
19.6	200	40	0.75	11.5	0	79.0	0.71	55.2	29.5	42.6	13.1
19.6	200	15	1.0	11.3	0	71.4	0.69	42.2	34.8	44.3	12.1
19.6	180	40	1.0	11.8	0	74.0	0.72	40.8	39.4	46.1	12.1
19.6	180	15	1.2	11.5	0	77.5	0.77	38.2	42.2	45.1	10.6
19.6	160	40	2.0	11.6	10	77.6	0.66	37.4	41.2	42.8	11.0
19.6	160	15	1.6	12.0	15	77.0	0.74	39.1	38.9	44.9	11.0
19.8	150	40	2.6	11.9	85	75.6	0.60	37.3	42.2	43.0	11.1
19.8	150	15	1.0	12.4	60	73.9	0.71	39.0	42.5	44.8	11.1
19.8	140	40	3.2	11.2	90	79.4	0.68	40.4	42.9	43.6	10.8
19.6	140	15	2.16	11.5	90	75.5	0.65	42.5	37.2	42.5	11.9
19.6	130	40	4.0	11.4	95	78.3	0.65	36.5	42.9	42.5	11.2
19.8	130	15	3.0	11.4	90	78.1	0.74	42.6	36.9	42.9	11.4
19.6	120	40	5.9	11.4	97	76.6	0.69	41.5	38.8	40.5	11.4
19.8	120	15	3.9	11.6	95	84.0	0.87	96.0	35.4	29.4	11.5
19.8	Ambient air			12.0	93	79.2	0.75	33.8	46.7	41.6	11.9

TABLE II  
ANALYSES OF VARIANCE FOR STARCH RECOVERY AS Affected BY DRYING CONDITIONS  
FOR THE YEARS 1952 THROUGH 1956, INCLUSIVE

SOURCE OF VARIATION	1952		1953		1954		1955		1956	
	df	M.S.	df	M.S.	df	M.S.	df	M.S.	df	M.S.
Total	25	.....	29	.....	28	.....	28	.....	29	.....
Control vs.treated	1	60.24*	1	18.82	1	6.38	1	10.22	1	33.72
Controls:		%		%		%		%		%
high-moisture vs. low-moisture	1	0.00	1	2.42	1	1.80	1	21.12	1	7.22
Temperatures	5	7.61	6	56.95**	6	36.45**	6	24.60*	6	52.21*
Moistures	1	16.83	1	32.79	1	21.96**	1	128.14**	1	64.20
Relative humidity	1	13.35	1	37.26	1	2.89	1	31.50*	1	8.04
Pooled error	16	4.93	19	12.00	18	3.27	18	7.06	19	19.33

TABLE III  
ANALYSES OF VARIANCE FOR PROTEIN IN STARCH AS Affected BY DRYING CONDITIONS  
FOR THE YEARS 1952 THROUGH 1956, INCLUSIVE

SOURCE OF VARIATION	1952		1953		1954		1955		1956	
	df	M.S.	df	M.S.	df	M.S.	df	M.S.	df	M.S.
Total	25	.....	29	.....	28	.....	28	.....	29	.....
Control vs. treated	1	0.0014	1	0.0035	1	0.0368	1	0.0250	1	0.0010
Controls:		%		%		%		%		%
high-moisture vs. low moisture	1	.0000	1	.0000	1	.0002	1	.0968	1	.0004
Temperatures	5	.0080	6	.0880**	6	.0602	6	.0712	6	.0254
Moistures	1	.0590*	1	.1119*	1	.1873*	1	.0343	1	.0761*
Relative humidity	1	.0376*	1	.0201	1	.0246	1	.0448	1	.0052
Pooled error	16	0.0077	19	0.0208	18	0.0305	18	0.0375	19	0.0131

aware of wide variability in processing characteristics of corn from different harvests. Seasonal effects were also apparent in earlier work on dried corn (4). Drying temperatures affected both subsequent starch recovery and separation of starch and protein. The means for these two processing characteristics were separated by Duncan's Multiple Range Test (2), and these results are presented in Table V. Starch recovery associated with 200°F. (93.3°C.) temperature was significantly lower than any of the other temperature treatments. The 180°F. (82.2°C.) temperature mean could be separated from the means of samples treated at 120° and 130°F. (48.9° and 54.6°C.). The intermediate temperature ranges resulted in considerable overlap, and the means associated with these temperatures could not be separated statistically. The protein remaining in starch at a drying temper-

TABLE IV  
ANALYSES OF VARIANCE FOR STARCH RECOVERY AND PROTEIN IN  
STARCH FOR THE YEARS 1953 THROUGH 1956  
(Combined analyses in percent)

SOURCE OF VARIATION	df	MEAN SQUARES	
		%	%
Total	117		
Years	3	989.72**	.02962**
Control vs. treated	1	62.95*	.0038
Controls: high-moisture vs. low-moisture	1	25.92	.0190
Temperatures	6	137.22**	.1620**
Moistures	1	47.06*	.0329
Relative humidity	1	4.09	.0060
Temperature $\times$ moisture	6	6.30	.0308
Temperature $\times$ R.H.	6	28.17*	.0106
Moisture $\times$ R.H.	1	0.28	.1249*
Temperature $\times$ moist. $\times$ R.H.	6	6.34	.0044
Error (year $\times$ treatment)	85	12.03	0.0303

TABLE V  
MEAN PERCENTAGE OF STARCH RECOVERY AND MEAN PERCENTAGE OF PROTEIN IN STARCH ASSOCIATED WITH DRYING TEMPERATURES

DRYING TEMPERATURES	°F	°C	STARCH	PROTEIN IN
			RECOVERY *	STARCH *
Control	Control		83.10 a	0.836 ab
120	48.9	82.45 a	0.836 ab	
130	54.6	82.44 a	0.741 a	
140	60.0	80.41 ab	0.807 a	
150	65.6	81.71 ab	0.837 ab	
160	71.1	80.87 ab	0.801 a	
180	82.2	79.46 b	0.958 bc	
200	93.3	74.03 c	1.032 c	

\* Any pair of means not followed by the same letter are significantly different at the 0.05 level of probability.

ature of 200°F. (93.3°C.) was significantly higher than any of the other treatments except that at 180°F. (82.2°C.). The 180°F. temperature could be separated from 130°, 140°, and 160°F. (54.6°, 60°, and 71.1°C.). These, however, were not statistically different from 120° and 150°F. (48.9° and 65.6°C.).

Initial moisture difference was significant for starch recovery. Low initial moisture resulted in a mean of 80.8%, whereas the mean for high initial moisture was 79.6%. Although this difference was found to be statistically significant it is probably of little practical importance, especially when compared to the temperature effects. As pointed out previously, protein in starch as affected by initial moisture was not consistent from year to year and consequently could not be considered a

real effect in the combined analysis. Relative humidity did not appear effective for either of the processing characteristics.

Two interactions were apparent in the combined analyses. A slight temperature by relative humidity interaction was evinced for starch recovery. This interaction can be observed from the means presented in Table VI. Mean differences associated with relative humidity were

TABLE VI  
MEAN PERCENTAGE OF STARCH RECOVERY ASSOCIATED WITH DRYING  
TEMPERATURES AND RELATIVE HUMIDITY

DRYING TEMPERATURE °F	°C	RELATIVE HUMIDITY	
		40%	15%
120	48.9	81.26	83.64
130	54.6	82.64	82.25
140	60.0	83.01	77.81
150	65.6	82.60	80.81
160	71.1	81.35	80.39
180	82.2	78.30	80.62
200	93.3	73.55	74.51

not consistent at all drying temperatures. In spite of this, it is obvious that the greatest effect observed was in the reduction of starch recovery associated with 200°F. (93.3°C.) drying temperature, regardless of the relative humidity. The other interaction was found between initial moisture and relative humidity in the separation of protein from starch. This interaction is shown in the table below, where it can be

Initial Moisture	Relative Humidity	
	40%	15%
High (30%)	0.902	0.850
Low (20%)	0.801	0.882

seen that high-moisture corn resulted in better protein-starch separation when dried at 15% R.H., whereas low-moisture corn dried at 40% R.H. rendered the more favorable results. Again these differences, though statistically significant, are quite small and may be of limited practical importance.

The analysis of variance of the viability test is presented in Table VII. Temperatures of 180° and 200°F. (82.2° and 93.3°C.) were not included in this analysis, as these extreme treatments rendered the seed nonviable in all years under test. As evident from Table VII, all main effects were significant, with the exception of relative humidity. A temperature by moisture interaction was also significant. It can be seen from Table VIII that this interaction has resulted primarily from

TABLE VII  
ANALYSIS OF VARIANCE OF VIABILITY AS AffECTED BY DRYING CONDITIONS  
(In percent)

SOURCE OF VARIATION	df	MEAN SQUARES
Total	109	
Years	4	1,690**
Control vs. treated	1	14,098**
Controls: high-moisture vs. low-moisture	1	1,488*
Temperatures	4	14,947**
Moistures	1	32,544**
Relative humidity	1	16
Temperature $\times$ moisture	4	1,607**
Temperature $\times$ R.H.	4	85
Moisture $\times$ R.H.	1	55
Temperature $\times$ moisture $\times$ R.H.	4	220
Error (year $\times$ treatment)	84	243

TABLE VIII  
MEAN PERCENTAGE VIABILITY ASSOCIATED WITH DRYING  
TEMPERATURE AND INITIAL MOISTURE

TEMPERATURE °F	TEMPERATURE °C	INITIAL MOISTURE		TEMPERA- TURE MEANS <sup>a</sup>
		High (30%)	Low (20%)	
Control	Control	72.2	96.6	84.40 a
120	48.9	56.9	90.4	73.65 ab
130	54.6	41.8	86.6	64.20 b
140	60.0	25.4	78.0	51.70 c
150	65.6	7.9	51.0	29.45 d
160	71.1	2.9	9.3	6.10 e

<sup>a</sup> Any pair of temperature means not followed by the same letter are significantly different at the 0.05 level of probability.

a differential magnitude of response rather than from any reversal effects. It is also apparent that the viability is affected by high temperature more seriously in corn of high initial moisture than in corn of low initial moisture.

The means of viable seed associated with drying temperatures are given in Table VIII. Significant differences were found between all pairs of means except those dried at 120° and 130°F. (48.9° and 54.6°C.). The percentage of viable seed was drastically reduced at temperatures above 140°F., especially when the initial moisture content of the corn was about 30%. The mean for the high initial moisture was 26.98% as compared with 63.06% for the low initial moisture.

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## WATER PENETRATION AND INTERNAL CRACKING IN TEMPERED WHEAT GRAINS<sup>1</sup>

GORDON M. GROSH AND MAX MILNER

### ABSTRACT

Studies of water penetration in wheat grains, carried out by means of X-ray and a novel freeze-sectioning technique which does not affect the moisture status and physical structure of the kernels, revealed that cracks, radial and transverse to the crease, occur in hard vitreous endosperm in advance of water movement through the kernels. These cracks are most noticeable at moisture levels in the range employed in commercial tempering operations.

Peripheral absorption of water creates stresses between wet and dry portions which cause radial and transverse cracks that provide pathways for further penetration of water. These fractures eventually facilitate the formation of middlings during milling. The cracking phenomenon is not observed in nonvitreous wheats.

In the past thirty years, considerable information concerning the mode of water penetration into the wheat kernel has been collected. At temperatures below 50°C., water enters the wheat kernel in the attachment region near the germ tip and within minutes wets the outer bran layers beyond the testa and the germ (4,6,8,11). The testa inhibits water movement from the bran into the endosperm (7), and moisture thus moves from the germ to the nucellar layer. Moisture movement into the endosperm is at a much slower rate than in the outer layers

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during the first minutes after wetting (1,8). Gehle (5) postulated that water penetrates into the endosperm by means of capillary action in air spaces existing between the bran and endosperm, as well as through cracks in the endosperm. However, no direct evidence has been presented in support of this theory, and for this reason, this theory of water penetration has been criticized by Schäfer (13).

The presence of internal fissures in naturally weathered wheat as well as in artificially dampened and dried grains was noted by Milner and Shellenberger (10), using radiographic techniques. Campbell and Jones (2) noted radial cracks in Manitoba wheat when it was dampened to 17% moisture and then dried. They deduced from observations of density change that relatively coarse-grained cracking might occur in the endosperm of vitreous grains during moistening, but, since these cracks could only be observed visually after drying, they suggested that these cracks were only partially developed during moistening and were widened in subsequent drying. In grain wetted beyond 20%, they associated the development of endosperm mealiness with fine-grained cracking.

These observations prompted a study to determine if this cracking phenomenon could be detected during the tempering process prior to the milling of wheat. Another objective was to clarify the mechanism of moisture penetration, particularly with respect to movement in the endosperm in relation to any cracking which might take place.

#### Materials and Methods

Samples of hard red spring wheat (varieties Lee and Selkirk), hard red winter wheat (variety Ponca), durum wheat (variety Mindum), and soft red wheat (variety Brevor) were supplied by the experiment stations at Minot, North Dakota, Manhattan, Kansas, and Wooster, Ohio. Ponca and Lee wheat varieties were used in most of this study.

*Radiographs.* Radiographs of wheat samples were obtained by the technique of Milner, Lee, and Katz (9), using a Picker industrial X-ray unit with a Machlett water-cooled beryllium-window tube operated at 15 ma. and at potentials of from 15 to 25 kv. Exposures were made on Kodak Type M industrial X-ray film processed according to the manufacturer's instructions.

*Sectioning Procedure.* A simple sectioning technique has been developed, utilizing a freezing microtome to permit preparation of slices of wheat kernels which can be examined microscopically by reflected light. By means of this procedure the movement of moisture during handling can be arrested at any stage, and any alteration in the internal physical structure of the wheat kernel is kept to a minimum. Sec-

tions of dry kernels could be cut in any plane or angle without producing signs of cracking or endosperm breakup. Regardless of the orientation of the knife's cutting surface to the wheat kernel, cracks in wetted kernels appeared, radial and transverse from the crease. These observations provided evidence that a minimum of alteration in the physical structure of the grain was produced by the sectioning technique. The method does not wet the section or change the location of moisture, and is useful for a range of conditions from soft wet kernels to hard dry kernels.

The wheat kernel is placed on the microtome stage in the desired plane relative to the blade (Fig. 1) and embedded in 25% aqueous warm gelatin solution. This medium was chosen because it possessed the desired liquid properties for embedding and yet was of sufficient concentration to prevent transfer of water from the gelatin to the sec-

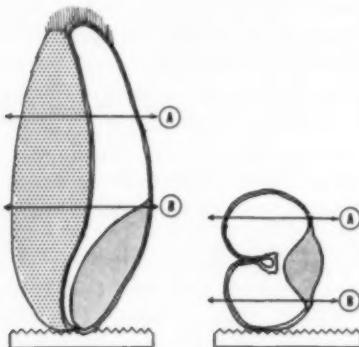


Fig. 1. Diagrammatic sketch showing orientation of wheat kernels on stage of freezing microtome for preparation of transectional slices (left) and longisectinal slices (right).

tioned material. This fact was confirmed by noting that at this concentration transfer of water to the sectioned surface could not be detected by the iodine vapor staining technique. After rapid freezing (15–30 seconds) with successive blasts of compressed carbon dioxide, slices not more than 15 microns thick are removed until a suitable plane surface is reached, 0.7 to 1.5 mm. above the surface desired to be examined (plane A, Fig. 1). With the tip of a hot electric soldering iron applied to the microtome stage, the gelatin base is melted without thawing of the kernel itself. The kernel slab is then inverted on the microtome stage, secured with several additional drops of liquid gelatin solution, and refrozen. The frozen kernel is sectioned down to the surface

desired to be examined (plane B, Fig. 1) by removing successive slices 10–20 microns in thickness. The microtome stage is warmed again with the soldering iron, and the gelatin is separated from the wheat slice before the latter has thawed. In this manner wheat slices with parallel surfaces are prepared. The section is placed in the iodine vapor chamber (see Staining Techniques) before it is completely thawed. Finally the slices are examined microscopically and photographed immediately after sectioning and staining.

*Staining Techniques.* The iodine vapor technique suggested by Ugrimoff (15) proved to be the most satisfactory means for mapping the movement of water in wheat. By placing wheat gluten, starch, and patent flour in chambers whose atmospheres range in relative humidity from 25 to 100% until hygroscopic equilibrium was reached and then staining these materials with iodine vapor, it was observed that a purple color developed in starch, and a brown color developed in gluten at moisture contents above 17%. Below 16% moisture, the surfaces stained yellow.

Bromophenol blue in benzene solution (1%) produced a purple color in gluten above 16% moisture, whereas it did not stain the starch portion of the endosperm.

*Hardness Test.* Because of the probable importance of hardness in relation to milling quality, a modification of the manufacturer's recommended technique (14) using the MIAG Micro-Hardness Tester was used. This tester is essentially a penetrometer which produces an indentation on the surface of the material to be studied by means of a wedge-shaped diamond under a controlled load. The size of the indentation provides a relative measure of the hardness of the material. Slices prepared with the freezing microtome are secured with rubber cement to the steel testing blocks and placed on the movable platform. Longisectional slices are oriented so that the impression of the diamond wedge is made in the long dimension of the kernel. The platform is slowly raised so that 2 seconds elapse before the catch on the handle is engaged. The diamond point, weighted with 1000 g., is allowed to rest on the kernel surface for 1 minute. The platform is then gently released and lowered. Impressions in the endosperm surface are read at 30 $\times$  magnification, with the use of a dissecting binocular having a calibrated scale in the eyepiece. One scale unit corresponds to 0.05 mm.

Preliminary statistical analyses on eight varieties of hard red winter wheats grown at eight experiment stations revealed that, according to the F test, 10 to 15 determinations per factorial cell, and 25 determinations in nonfactorial experiments, were needed to detect differences

significant to two integers. The higher the hardness value, the softer the surface.

*Wetting Techniques and Incubation.* In the wetting studies where wheat was exposed to an excess of moisture, the kernels were completely immersed in water at 75°F. for 30 seconds. After removal from the water they were placed on a sheet of moistened blotting paper with the crease side of the kernel adjacent to the paper. The kernels on the blotting paper were kept in a covered watch-glass in a room at 75°F. until sectioned.

When kernels were tempered to a predetermined moisture content, 3 g. of wheat were weighed into a glass-stoppered test tube. The water needed to raise the wheat to the desired moisture content was added to the kernels by means of a hypodermic syringe calibrated to 0.01 ml. The tube was stoppered and gently shaken for 5 minutes. The kernels were kept in sealed test tubes until examined.

### Results

*Radiographic Studies.* Samples of hard red spring (Lee) wheat were wetted to 17 and 21% moisture, sealed in a plastic orientation tray, and radiographed by means of X-rays, 0, 0.5, 1, 2, 4, 8, 12, and 24 hours after wetting. Prior to wetting, kernels were radiographed and those which exhibited any sign of cracking visually or radiographically were not used in the subsequent wetting experiments. Although no cracks were noted in those kernels studied prior to wetting, transverse and radial cracks could be noted within 30 minutes after wetting (Fig. 2, top and middle). After 8 hours' standing, these cracks could no longer be observed (Fig. 2, bottom). The apparent disappearance of cracks in the radiographs was probably due to a swelling of the endosperm and hence a decrease in the intercrack spaces to dimensions below the resolving power of X-rays (10).

*Wetting Patterns in the Presence of Unlimited Moisture.* Samples of hard red spring (Lee) and hard red winter (Ponca) wheats were wetted in the presence of an excess of moisture, sectioned, and stained with iodine vapor 0, 0.5, 1, 2, 4, 8, 12, 18, and 24 hours after wetting. At zero time, sections of unwetted kernels revealed no sign of iodine staining or cracking as a result of sectioning (Fig. 3, A and E; Fig. 4, G). Thirty minutes after wetting a slight darkening below the germ tip was observed. At this time cracks, radial and transverse from the crease, were already evident as they began to form at the germ end (Fig. 4, I). Sandstedt (12) has suggested that endosperm breaks up most readily along the cell wall. Observations at the initiation of cracking of freeze-sectioned slices also showed that cracks occurred along lines

which resemble the cellular structure of the endosperm. Within 1 hour after wetting, the cracks radial and transverse to the crease extended over the entire wheat kernel (Fig. 3, B and F; Fig. 4, A and D). A concentration of moisture in the cracks at the germ end was noted ahead of the general migration of moisture through the endosperm (Fig. 4, B).

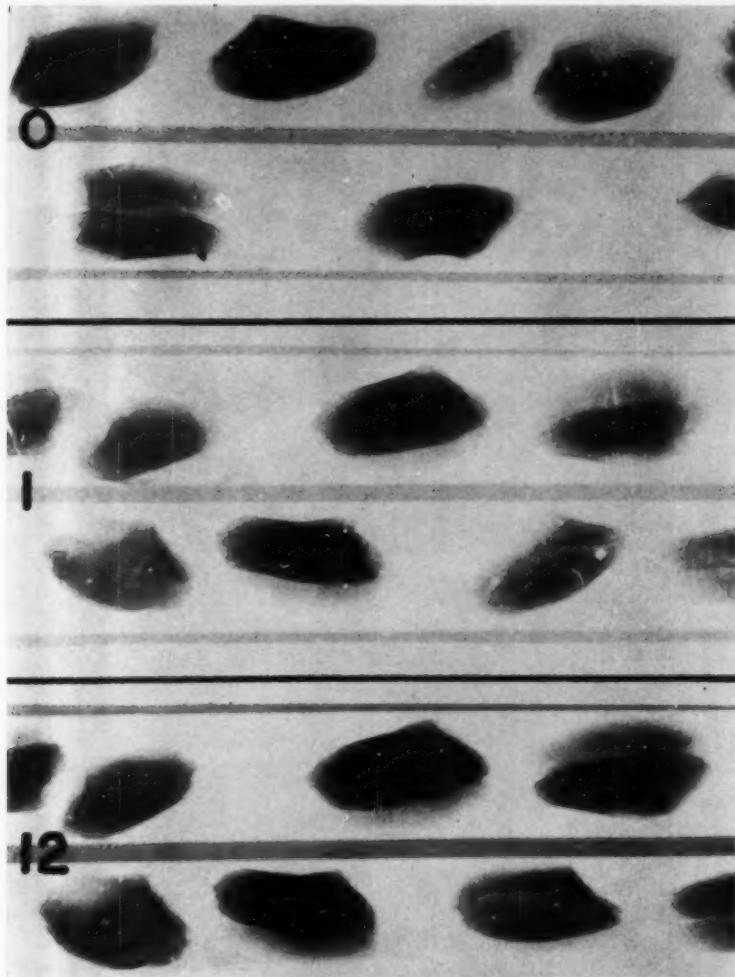


Fig. 2. Radiographs of hard red spring wheat (Lee variety) tempered to 17% moisture 0, 1, and 12 hours after wetting.

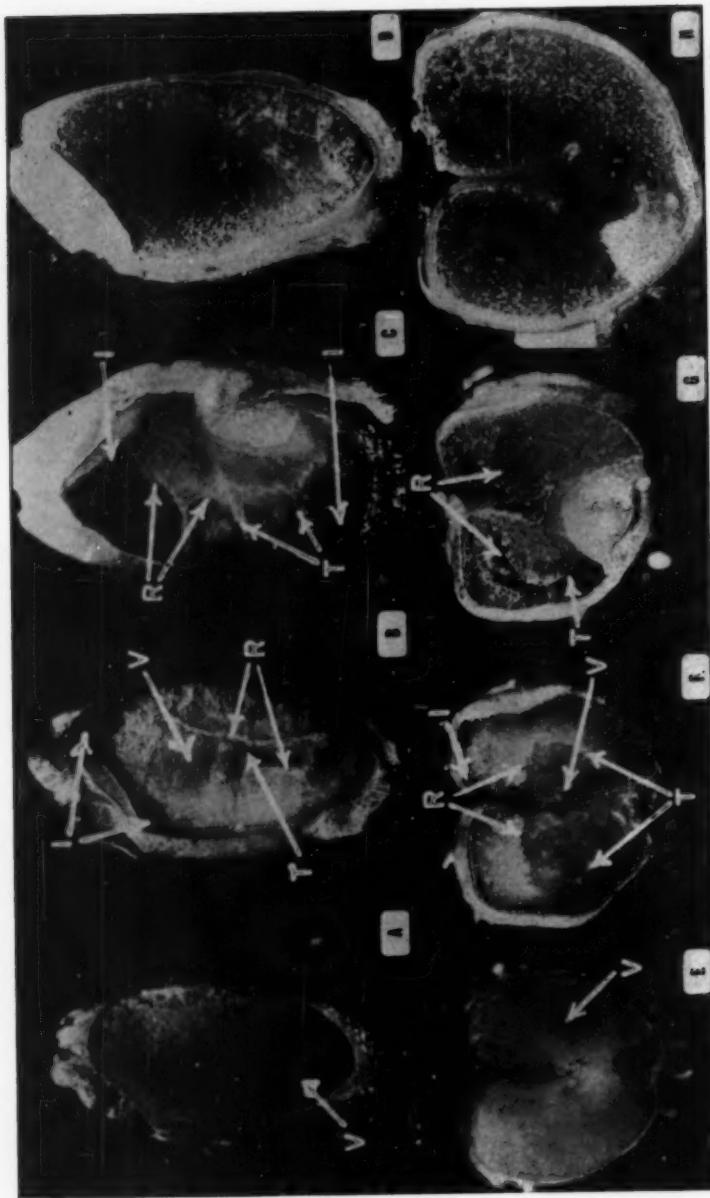


Fig. 3. Macrophotographs of iodine-stained slices of hard red spring wheat wetted in an excess of water. Longisectinal slices 0 hours (A), 1 hour (B), 4 hours (C), and 8 hours (D) after wetting. Transsectinal slices 0 hours (E), 1 hour (F), 4 hours (G), and 8 hours (H) after wetting. R: radial cracks; T: transverse cracks; I: darkening due to iodine stain; V: darkening due to vitreous endosperm.

Although previous workers have claimed a slow, even movement of moisture from the germ end and aleurone layer to the endosperm interior, these sections taken from 1 to 4 hours after wetting indicated a sectional wetting varying in intensity in different particles bounded by these cracks (Fig. 4, B and H). Three and four hours after wetting, the moisture had migrated over the entire endosperm, but a concentration of moisture near the cracks was evident (Fig. 3, C; Fig. 4, C, E, and J). Sections taken 8 hours after wetting indicated that there was then an over-all and more even distribution of moisture through the entire endosperm (Fig. 3, D and H; Fig. 4, F), with a higher moisture content at the germ and crease regions. Cracks were less evident as the moisture became more evenly distributed. Twelve hours after un-

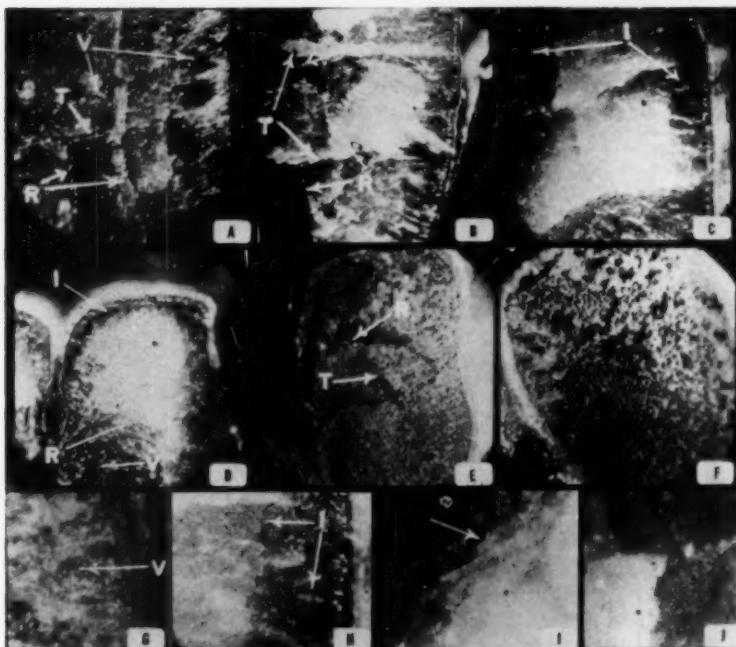


Fig. 4. Photomicrographs of iodine-stained slices of hard red spring wheat wetted in an excess of water. Longisectional slices 1 hour (A), 2 hours (B), 4 hours (C) after wetting ( $60\times$ ). Transectional slices 2 hours (D), 4 hours (E), and 8 hours (F) after wetting ( $60\times$ ). Longisection of endosperm in center dorsal region prior to wetting (G) and 4 hours after wetting (H). I — Transection through endosperm center 1 hour after wetting, showing initiation of crack. J — longisection through endosperm center 4 hours after wetting, showing uneven moisture distribution and cracking ( $150\times$ ). V: darkening due to vitreous endosperm; I: darkening due to iodine stain; R: radial cracks, T: transverse cracks.

limited wetting, the cracks could no longer be detected in the endosperm.

The cracking pattern was similar in both hard red winter (Ponca) and hard red spring (Lee) wheats. Staining studies using bromphenol

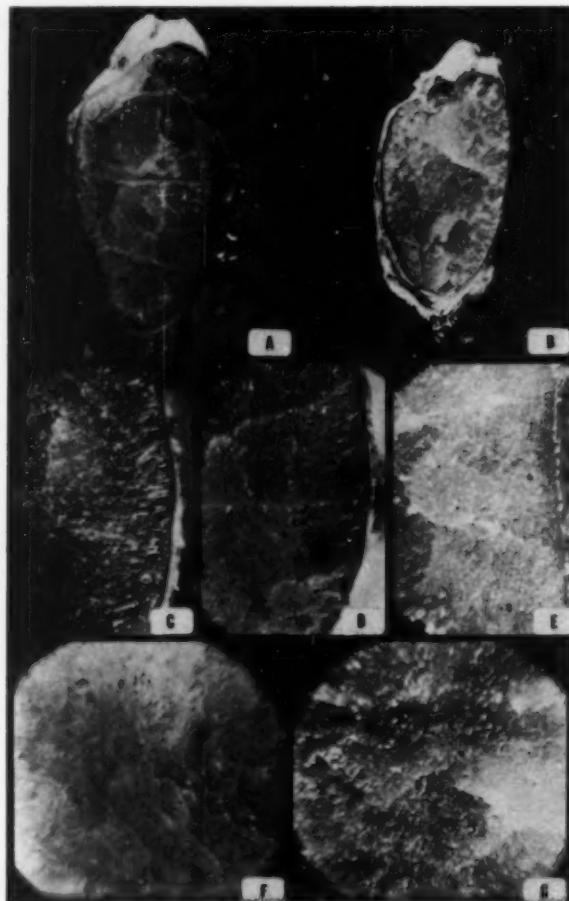


Fig. 5. Macrophotographs of longisectinal slices (iodine-stained) of hard red spring wheat tempered to 17% moisture, 4 hours (A) and 24 hours (B) after wetting. Photomicrographs of longisection of dorsal endosperm 2 hours (C), 4 hours (D), and 24 hours (E) after wetting (60 $\times$ ). F—transection 2 hours after wetting showing concentration of moisture along cracks. G—longisection 4 hours after wetting through center endosperm, showing uneven moisture distribution with concentration at cracks (150 $\times$ ).

blue yielded results which confirmed this wetting pattern, although it was not possible to make any observations concerning cracking because of the liquid nature of the stain which altered the physical condition of the endosperm surface. Both hard red spring and hard red winter wheat varieties showed similar wetting patterns when a bromphenol blue stain was used.

*Behavior at Tempering Moistures.* To observe the mode of water penetration at moisture contents comparable to those used in the tempering process in milling, samples of hard red spring (Lee) and hard red winter (Ponca) wheats were wetted to 17% moisture, sectioned, and studied. Since the moisture content was too low to show marked iodine vapor staining, the movement of moisture was not readily observed except under high magnification. Cracks radial and transverse to the crease were observed within the first hour after wetting (Fig. 5, A and C). It was possible to see a movement of moisture along these cracks, particularly along those which had formed radially to the crease (Fig. 5, F and G). A sectional wetting of the wheat in fragments bounded by these cracks could be observed (Fig. 5, A, D, and G). Eight hours after wetting, a relatively even moisture distribution throughout the kernel had taken place. The cracks which presumably had formed during the first hour of wetting were still evident in the kernels sectioned even after 24 hours' wetting (Fig. 5, B and E).

Samples of Ponca wheat wetted to 15.5, 17.5, and 18.5% moisture content revealed that a similar cracking pattern could be observed at all three moisture levels. Eight hours after wetting, a proliferation of secondary cracks arising from the radial and transverse primary cracks was noted, particularly at 15.5% moisture content. At moisture values above 17%, it was observed that these secondary cracks were not noticeable 8 hours after wetting. This may be due to the greater swelling of the endosperm at higher moistures. When wetted to 20% moisture, these primary cracks were no longer visible 12 hours after wetting. Kernels wetted to only 13% moisture showed very little, if any, cracking upon sectioning.

Changes in hardness with moisture and time of Ponca wheat are reported in Table I. At all moisture values studied, the kernels reached a maximum softness 12 hours after wetting and then the hardness value returned to the level reached between 4 and 8 hours following initial wetting. As was expected, the higher the moisture content, the softer the resulting tempered wheat.

*Behavior of Different Wheat Classes.* Varieties of hard red spring, hard red winter, durum, and soft wheats were tempered to 17% moisture and sectioned at various times after wetting. The behavior of hard

TABLE I  
EFFECT OF TEMPERING MOISTURE ON ENDOSPERM HARDNESS OF HARD RED WINTER (PONCA) WHEAT

TIME AFTER WETTING	TEMPER MOISTURE (PERCENT) AND HARDNESS VALUE <sup>a</sup>		
	15.5	17.0	18.5
<i>hours</i>			
0 <sup>b</sup>	5.8	—	—
1	5.3	7.5	6.6
4	5.7	7.4	7.7
8	7.5	9.3	9.3
12	7.6	9.5	10.4
24	6.7	8.0	8.7

<sup>a</sup> Mean of 15 determinations.

<sup>b</sup> Unwetted.



Fig. 6. Macrophotographs of longisectinal slices of soft (A,B,C), durum (D,E,F), and hard red winter (G,H,I) wheats tempered to 17% moisture and photographed prior to wetting (A,D,G); and 1 hour (B,E,H) and 24 hours (C,F,I) after wetting.

red spring wheat has already been illustrated (Fig. 5). Although the durum wheat exhibited the same primary cracking pattern as did the hard red winter and hard red spring wheats (Fig. 5, A; Fig. 6, E and H), a much more extensive degree of secondary cracking after 4 hours' standing was noted (Fig. 6, F) than in either of the hard red wheat samples studied (Fig. 5, B; Fig. 6, I). No cracking could be observed in the soft white wheat (Fig. 6, A, B, and C). It should be noted that in hard wheat samples which were not vitreous (mealy kernels), this cracking pattern was not observed.

All four wheat classes studied exhibited a maximum softening 12 hours after wetting and then returned to the hardness observed between 4 and 8 hours after wetting (Table II). Although durum wheats

TABLE II  
CHANGE IN ENDOSPERM HARDNESS IN FOUR WHEAT TYPES TEMPERED TO 17% MOISTURE

TIME AFTER WETTING	WHEAT CLASS AND HARDNESS VALUE <sup>a</sup>			
	Soft (Brevor)	Durum (Mindum)	HRS (Selkirk)	HRW (Ponca)
<i>hours</i>				
0 <sup>b</sup>	9.8	5.9	5.6	5.8
1	11.0	6.7	6.1	7.5
4	11.4	9.0	8.9	7.4
8	12.4	8.7	9.5	9.3
12	12.0	8.4	9.3	9.5
24	11.8	9.1	8.8	8.0

<sup>a</sup> Mean of 15 determinations.

<sup>b</sup> Unwetted.

are popularly considered to be harder than either the hard red winter or hard red spring wheats, the hardness values obtained in this study for the three hard wheats were very similar.

### Discussion

Evidence is presented here to indicate that cracks are initiated in wheat by the tempering process. The X-ray studies confirmed the presence of transverse and radial primary cracks. Observations of the concentration of moisture at the cracks in the early stages of wetting and the sectional wetting of endosperm portions bounded by these cracks provide additional evidence that these cracks are formed in the kernel as a result of tempering and do not result from the handling or sectioning techniques. These observations agree with Gehle's (5) postulate that water moves into the endosperm through cracks, although no evidence has yet been presented to prove that the movement of this moisture along the cracks is of capillary nature.

Although other workers have noted cracking when endosperm is

dried (2,10), direct evidence that cracks form in wheat as a result of wetting of endosperm alone (tempering) has not been offered previously. While it would be reasonable to assume that some fine cracking may exist in sound grain, these results indicate a pronounced increase in cracks, both as to number and dimension, as a result of wetting. The forces causing cracking as a result of tempering might have two sources: a residual stress set up within the wheat endosperm during the maturing stage of kernel development, and a gradient of swelling forces produced when the moisture is absorbed into the wheat kernel. In this connection, Milner and Shellenberger (10) found that rapid drying of immature grain of high moisture content does not produce fissuring, but such dried grain when rewetted and redried fissures readily, indicating a fundamental change in structure of the endosperm constituents with maturation. It is possible that the internal stresses of natural maturing are of sufficient magnitude to cause cracking by release of these stresses, but in most kernels the internal stresses produced by maturing are not released until a moisture gradient is established. Earle and Ceaglske (3), in a study of factors causing checking in macaroni, presented evidence as to the mechanics of cracking due to stresses arising from unequal moisture gradients produced by the addition of moisture. Natural weathering (wetting and drying) or grain tempering prior to milling creates an additional internal endosperm stress which, when added to the maturing stress, causes the stress within the endosperm to exceed the breaking point, thus producing cracks. Secondary cracks, arising from the primary cracks, radial and transverse from the crease at moisture levels used in tempering, break up the endosperm into particles in the size range of wheat middlings. The fact that at moisture levels corresponding to those used by millers in tempering, primary and secondary cracking is very evident and persists during the tempering period, suggests that middlings may be preformed in the actual tempering operation which facilitates their separation in the break process of milling. These cracks in vitreous wheats clearly provide a pathway for movement of water into the endosperm during the cold tempering process. However, in the tempering of soft or mealy kernels, the cracking phenomenon is of no significance.

Further work is needed to assess the practical implication of cracking in the tempering process. Studies of the mechanical properties of wheat endosperm and the mechanisms causing cracking would be of great value in understanding the physical effects of tempering in the milling process.

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## GAS EXCHANGE INDUCED IN DRY WHEAT EMBRYOS BY WETTING<sup>1</sup>

PEKKA LINKO AND MAX MILNER<sup>2</sup>

### ABSTRACT

Exposing "dry" wheat embryos to water vapor causes an immediate enzymatic production of carbon dioxide. This gas evolution is further increased by complete wetting of the germ material. The optimum pH of this reaction is 5.8. The addition of pyruvate markedly increases carbon dioxide evolution, but slightly inhibits oxygen uptake, thus resulting in significantly elevated respiratory quotient values. Alpha-ketoglutarate also increases carbon dioxide evolution a little. Malonate strongly inhibits these reactions.

These results point to the major role of tricarboxylic acid cycle intermediates and compounds closely related to them in the biochemical organization associated with the viability and storage stability of grains.

Moisture content and temperature are the cardinal factors affecting the viability and biochemical stability of stored seeds and grains. The respiration of sound dry grains (moisture values which are in equilibrium with relative humidities at or below about 65%) is of an extremely low order in comparison with that produced by vegetative saprophytic fungi, whose spores occur on and frequently within the coat of grains, and which grow slowly at relative humidity levels as low as 65% and rapidly when the interseed relative humidity exceeds 75%. The rapid loss in viability and biochemical quality suffered by grain stored under adverse moisture conditions favorable to fungal growth, but below that required for seed germination, has been related largely to toxic products and enzymes secreted by storage fungi (6). Another type of storage-induced deterioration in wheat grains is a nonenzymatic browning reaction in embryos, which apparently involves proteins and reducing sugars (3). No adequate explanation has been offered, however, for the slower but significant decreases in viability as well as the biochemical changes occurring in grain stored at "safe" moisture levels, i.e. below those favorable to fungal growth, particularly at elevated environmental temperatures.

The present study was undertaken with the conviction that the keto acids and related biochemical compounds involved in the tricarboxylic acid cycle probably play an important role in the survival of seeds in storage. This theory proposes that the initial germinative processes in grains depend on a carry-over through the storage period of

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<sup>2</sup> Assistant Professor and Professor, respectively, Kansas State College.

limited quantities of one or more of these metabolic intermediates, and that these substrates, which are vital to the initial germinative process, are dissipated in storage at a rate depending upon the adversity of the storage environment in terms of elevated moisture content and temperature. It seems probable that no production of additional amounts of these metabolic substrates can occur in the grain until sufficient moisture is provided to initiate the relatively complete metabolism involved in germination. The depletion of these limited substrates during storage would, therefore, result in loss of viability and biochemical organization of the embryo tissue.

James (2) has written a comprehensive and penetrating review of the nature of plant respiration, in which he has evaluated the role of the tricarboxylic acid cycle and other respiratory mechanisms in this process. No information appears in the literature, however, concerning these relationships in stored grain, and very little with reference to seeds in the very early stages of germination.

In previous respiration studies with peas (1), soybeans (4), and wheat germ (3), it has been observed that dampening of the tissue to moisture levels below the germination requirement leads to an immediate but transitory release of carbon dioxide, which subsides many hours before the germination of fungal spores begins. These observations, as well as others on the characteristics of the gas exchange in spontaneously heating seeds (5), suggest that this evolution of carbon dioxide is due to an enzymatic process in seed or grain tissues, totally unrelated to the metabolism of fungi or other microorganisms.

In order to explore this possibility and to evaluate the relationship of this enzymatic decarboxylation to the subsequent properties of the grain, a quantitative study was undertaken, using Warburg manometric techniques, of the immediate effect of wetting of excised wheat embryos on their carbon dioxide evolution and oxygen uptake, and of the effect of tricarboxylic acid cycle acids on this phenomenon.

#### Materials and Methods

Fresh wheat embryo tissue (a by-product of certain milling processes) in a clean unprocessed granular form, supplied by International Milling Co., Minneapolis, Minnesota, was used in these studies. This material is essentially free of bran and starchy endosperm. Its moisture content was 9.2% (wet weight basis) and it was stored in a moisture-proof container at +4°C.

Assays were carried out in Warburg flasks according to the direct method as outlined by Umbreit *et al.* (8). Ethyl lactate colored with Crystal Violet was used as the manometer fluid. Potassium hydroxide

solution (20%), soaked into filter paper strips, was used in the center well to absorb carbon dioxide during determinations of oxygen uptake. One hundred milligrams of wheat germ were used for all determinations and 1 ml. of wetting medium was placed into the side arm. The flasks were incubated at 30°C. After the liquid was tipped from the side arm into the cell, the stopcocks were left open for 5 minutes, during which time the shaker was started (106 to 108 strokes per minute). The stopcocks were closed, and manometer readings were taken at 5-minute intervals, usually for 35 minutes.

Standard phosphate buffers 0.067M (*M/15*), were used in these studies. Buffered solutions of potassium salts of organic acids were used to determine the effect of these compounds on the gas exchange. The pH of these solutions was adjusted to 5.8.

The results were corrected for retention of carbon dioxide in buffer solutions.

### Results and Discussion

The results, presented in Table I, indicate that at 30°C., "dry" wheat germ produces readily measurable carbon dioxide evolution. This result was not unexpected in view of other observations concerning the marked biochemical lability of this material (7). Exposure of

TABLE I  
CARBON DIOXIDE PRODUCTION FROM 100-MILLIGRAM SAMPLES OF WHEAT GERM

TIME	"DRY" GERM	GERM PLUS WATER VAPOR	WETTED GERM
minutes	$\mu\text{l}$	$\mu\text{l}$	$\mu\text{l}$
5	2.0	16.7	18.2
10	1.7	26.4	32.3
15	2.7	31.9	47.2
20	2.8	37.5	61.1
25	3.9	40.2	76.0
30	4.0	44.4	89.9

the germ to water vapor increases carbon dioxide production tenfold, and complete wetting of the germ further considerably increases this gas evolution. The observation that carbon dioxide production can be increased by short-term exposure of the germ to elevated humidity may have considerable significance in rationalizing the effect of natural environmental fluctuations of humidity on gas exchange from commercially stored grain.

The results of this first study suggested that enzymatic processes are stimulated simply by increasing the moisture content in dry wheat germ. When 0.067M (*M/15*) phosphate buffers were used it was found

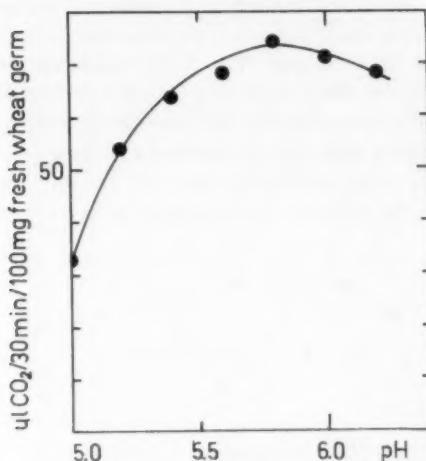


Fig. 1. Effect of pH on carbon dioxide evolution from wetted wheat germ.

that the pH optimum of this carbon dioxide evolution is close to 5.8 (Fig. 1). The effect of pH on the gas production supports the theory of the enzymatic nature of this reaction.

Figure 2 shows the effect of a number of tricarboxylic acid cycle intermediates and malonate on the oxygen uptake and carbon dioxide evolution from wetted wheat germ. The acids were used in concentrations of 0.000, 0.0125, 0.025, 0.050, and 0.100M.

Pyruvic acid was the only keto acid employed in these studies which produced a very marked increase in carbon dioxide production, the optimum lying at about 0.05M pyruvate. At this optimum condition, carbon dioxide values 2 to 3 times greater than the control were observed; at the same time, the oxygen uptake was somewhat inhibited. Thus very high respiratory quotient values appeared for the net gas exchange, reminiscent of the trend noted in the earlier respiration studies (1,3,4). Alpha-ketoglutarate also produced a moderate increase in carbon dioxide evolution at lower concentrations.

Malate, fumarate, succinate, and citrate all caused a slight increase in carbon dioxide production at concentrations between 0.0125 and 0.025M, but higher concentrations seemed to be somewhat inhibitory. Only in the case of 0.1M malate was the carbon dioxide evolution still above the control.

Malonic acid, which is a well-known inhibitor of the Krebs cycle, showed a regular and marked inhibition of both oxygen uptake and

carbon dioxide evolution. The inhibitory effect of malonate was further investigated with reference to the activation of carbon dioxide evolution induced by pyruvic acid (Fig. 3). It was found that malonate drastically reverses the effect of pyruvate on the carbon dioxide evolution, the 0.20M malonate virtually eliminating gas production.

This study suggests that wetting activates a decarboxylating mechanism which involves pyruvic decarboxylase and possibly another enzyme related to alpha-ketoglutarate. Additional studies have been initiated

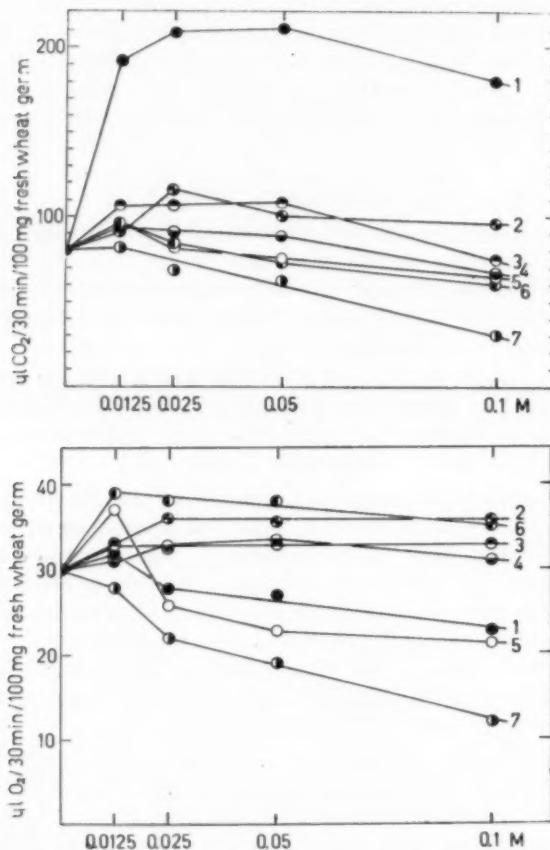


Fig. 2. Effect of some Krebs cycle acids at various concentrations on carbon dioxide evolution (top) and oxygen uptake (bottom) by wetted wheat germ. 1, pyruvate; 2, malate; 3, alpha-ketoglutarate; 4, fumarate; 5, citrate; 6, succinate; 7, malonate.

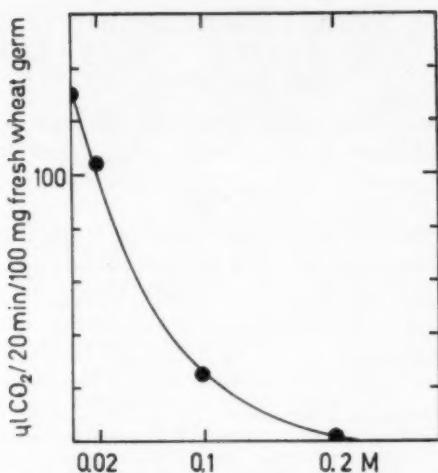


Fig. 3. Effect of malonate on carbon dioxide evolution from wheat germ in the presence of 0.1M pyruvate.

to determine whether other intermediates, including amino acids, are also involved. The relationship of this gas exchange to the biochemical deterioration of the grain is also under investigation.

#### Acknowledgment

The technical assistance of Miss Yu Yen Cheng is gratefully acknowledged.

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# FREE AMINO AND KETO ACIDS OF WHEAT GRAINS AND EMBRYOS IN RELATION TO WATER CONTENT AND GERMINATION<sup>1</sup>

PEKKA LINKO<sup>2</sup> AND MAX MILNER<sup>2</sup>

## ABSTRACT

The free amino and keto acids of mature wheat grains and excised embryos were determined by quantitative paper chromatography in relation to changes occurring during brief storage at various moisture contents and temperatures. Glyoxylic acid was the predominant keto acid in both resting seed and excised germ. Raising the water content of the germs to 18% caused a marked decrease in alpha-ketoglutarate and glutamate in only a few hours. After only 1 hour at 40% moisture content at room temperature, pyruvate became the predominant keto acid in excised germ. In 24 hours under these conditions, alpha-ketoglutarate, oxaloacetate, glutamate, and aspartate virtually disappeared from excised wheat germ; in contrast, most of the free amino acids increased, originating from proteins apparently by hydrolysis. During germination of intact seeds at 40% moisture content alpha-ketoglutarate, oxaloacetate, glutamate, and aspartate increased. Carbon dioxide and nitrogen atmospheres intensify the decrease in alpha-ketoglutarate, oxaloacetate, and corresponding amino acids from excised germs.

The data obtained suggest that carbon dioxide evolution observed from excised germ, either freshly wetted or exposed to elevated humidity, is due primarily to the decarboxylation of glutamic acid by a seed enzyme.

The reaction between amino acids released from proteins by enzymatic hydrolysis, with existing carbohydrates, may be the primary cause of non-enzymatic browning in wheat germ. Breakdown of proteins and loss of important tricarboxylic acid cycle intermediates catalyzed by seed enzymes stimulated by small increases in moisture content appear to be important initial causes of loss of viability in stored seeds. The later appearance of vegetative saprophytic fungi greatly aggravates the deteriorative process.

Although it is generally recognized that the respiration, heating, chemical deterioration, and loss of viability which occur in cereal grains stored under ordinary environmental conditions are due largely to certain saprophytic fungi (2,7,16,17,18,24), evidence exists that deleterious biochemical changes can occur in grain in advance of damaging fungal development. For example, it has been observed that dicotyledonous seeds (peas, soybeans) and embryos of wheat, when dampened only moderately to moisture values well below that required for seed germination, produce an immediate and sizeable carbon dioxide and oxygen exchange, characterized by elevated respiratory quotients (5,17, 18). This gas exchange recedes before germination of fungal spores can affect the subsequent respiratory pattern. That this excessive car-

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<sup>2</sup> Respectively, assistant professor and professor, Kansas State College.

bon dioxide/oxygen ratio involves metabolism of the seed itself rather than that of fungi is indicated by the fact that autoclave-sterilized seeds, inoculated with pure cultures of fungi which normally cause spontaneous heating, produce the same heating trends without the sharp rise of respiratory quotient (19). It has also been demonstrated that a typical Maillard or browning reaction occurs in the embryos of stored wheat in the absence of activity characteristic of mold growth (14).

These facts suggest that the immediate gas exchange associated with wetting of seed tissue is an enzymatic process totally unrelated to the metabolism of fungi or other microorganisms. Such observations have led the authors to propose that changes in the keto acids and related labile biochemical compounds involved in the tricarboxylic acid cycle probably play an important role in the survival of seeds in storage, and that depletion of these limited and chemically sensitive substrates, as might occur in storage, would injure the biochemical organization of the seed and therefore limit its storage stability and viability. A preliminary evaluation of this speculation in terms of the immediate effect of wetting of wheat embryos on their gas exchange, and the role of some Krebs cycle intermediates in this process, supports this theory (13).

These observations have prompted the present quantitative study of the immediate effects of wetting intact wheat grains and excised embryos, at different temperatures, on the free amino and keto acid content of these materials, and an exploration of the relationship of changes in these compounds to seed viability and biochemical deterioration in storage.

### Materials and Methods

**Materials.** Fresh granular wheat germ was obtained from International Milling Co., Minneapolis, Minnesota. Two different samples with moisture contents of 10.7 and 9.2% were used.

For experiments with grain, soft red winter wheat was used, variety "Seneca," grown in Ohio. The moisture content of the seeds was 11.6%, the total protein 9.9–10.4%, and germination above 95%. Both germ and grain were stored in sealed containers at +4°C.

**Moisture.** Moisture content (wet-weight basis) was determined by drying the samples for 1 hour at 130°C. Samples of different moisture contents were prepared by adding the calculated amount of distilled water with vigorous mixing, which in the case of gerin was continued for a few minutes after all the water was added, to achieve even moisture distribution.

**Determination of Keto and Amino Acids.** The technique for isolation of keto acids for quantitative determination was a modification

of methods developed by Virtanen *et al.* (1,33) and Towers *et al.* (29), and involves two-dimensional chromatographic separation of amino acids derived by catalytic reduction of the hydrazones of the keto acids extracted from the plant material. One hundred grams of material, mixed in a Waring Blender for a few minutes with 400 ml. of freshly mixed tungstate reagent,<sup>3</sup> were centrifuged. The same procedure was repeated twice with the precipitate and 200-ml. portions of the tungstate reagent. The clear supernatant solutions were immediately added to 100 ml. of a freshly prepared 1% solution of 2,4-dinitrophenylhydrazine (2,4-DNPH) in 5*N* sulfuric acid. After standing for 1 hour at room temperature, this reaction mixture was extracted with ethyl acetate and the 2,4-DNPH derivatives of keto acids produced were extracted by shaking with an aqueous 10% w/v solution of sodium carbonate and the pH of the extract adjusted to 1 with 5*N* sulfuric acid solution. The hydrazones were again extracted with ethyl acetate, and this extract was washed five times with 100-ml. portions of distilled water. The ethyl acetate solution was dried overnight with anhydrous sodium sulfate and vacuum-distilled to dryness. The residue was redissolved in a small amount of 95% ethanol, passed through a 3-ml. column of Amberlite IR-120 ( $H^+$ ) resin to remove possible traces of amino acids, and subjected to reduction.

The reduction of the hydrazones to amino acids was performed with Adams platinum oxide catalyst, by passing hydrogen through the reaction mixture fast enough to stir it thoroughly. Sometimes it was necessary to add a small amount of catalyst during the course of the reduction. The end point of the reduction could be detected conveniently by the disappearance of the color of the mixture. However, the aromatic nitroamines arising from the 2,4-DNPH part of the molecule are also colored, and the reduction of the nitro groups requires a considerable length of time. About 1 ml. of Amberlite IR-120 ( $H^+$ ) resin was therefore added to the reaction mixture to bind the amino compounds formed. After the reduction was complete (usually from 4 to 10 hours) the contents of the tube were poured into a small column containing about 1 ml. of Amberlite IR-120 ( $H^+$ ) resin, the column was washed with 95% ethanol, and the amino compounds were displaced by 1*N* ammonium hydroxide solution. Amino acids could easily be separated from most of the aromatic side products, but this treatment was not usually necessary since these side products do not interfere with subsequent separation and identification. The final yields calculated as pure keto acids were as follows: oxaloacetic

<sup>3</sup>  $H_2O$ , 1*N*  $H_2SO_4$ , and 10% w/v  $Na_2SO_4 \cdot 2H_2O$  in the proportions 3:1:1.

acid 35%, alpha-ketoglutaric acid 40%, pyruvic acid 70%, and glyoxylic acid 98%. These recovery values were used in calculating the keto acid content of the extracts.

Free amino acids were extracted from plant material with 70% (w/v) ethanol and purified by treatment with Amberlite IR-120 ( $H^+$ ) resin, and elution with 1N ammonium hydroxide solution.

Whatman No. 4 filter paper was used without prewashing for two-dimensional chromatography. The first solvent was *n*-butanol-acetic acid-water (63:10:27) and the second was phenol<sup>4</sup> saturated with water (ammonia atmosphere). Hydrocyanic acid was used to prevent the "pink front" of ninhydrin-sprayed amino acid spots. This sequence of solvents was found to be critical, because if the side products from the catalytic reduction of the 2,4-DNPH derivatives were not removed, phenol could not be used as the first solvent. These compounds did not interfere seriously even in relatively high concentrations when *n*-butanol-acetic acid-water was used first. The chromatograms were dried at room temperature and sprayed with a solution of 0.25% ninhydrin in 95% ethanol.

For keto acid determinations the entire extract from 100 g. of plant material was usually used for a single two-dimensional chromatogram. For chromatograms of free amino acids an extract aliquot equivalent to 100  $\mu$ g. of germ and 200–500  $\mu$ g. of grain was sufficient to give satisfactory results. The individual amino acids were identified by co-chromatography with known amino acids.

For quantitative determinations, the spots were first compared with a concentration series ranging from 1 to 400  $\mu$ g. of pure amino acids. The error during visual comparison was found to be within  $\pm 5$ –10%, depending on the concentration and individual amino acid. Colorimetric quantitative measurements were done according to Fischer and Dörfel (4), with the exception that two-dimensional chromatography was used.

### Results and Discussion

**Free Keto Acids.** As indicated by the data in Table I, glyoxylic acid is the predominant keto acid in both excised wheat germ and whole grain. Alpha-ketoglutaric and pyruvic acids are also present in significant quantities, but other keto acids occur in essentially trace amounts. Recently small amounts of glyoxylic, hydroxypyruvic, alpha-keto-beta-hydroxybutyric, and alpha-keto-gamma-hydroxybutyric acids have been shown in other plant material (28,30,31,35). There has been some doubt about the existence of natural hydroxypyruvate since Kulonen

<sup>4</sup> Merck's reagent grade phenol was used without redistilling.

TABLE I  
THE FREE KETO ACID CONTENT OF WHEAT GERM AND INTACT WHEAT GRAINS  
(Moisture-free basis)

KETO ACID	GERM	WHEAT GRAIN
	μg/100 g	μg/100 g
Glyoxylic	165	17
Alpha-ketoglutaric	70	1
Pyruvic	40	5
Oxaloacetic	5	1
Hydroxypyruvic	4	1
Alpha-keto-beta-hydroxy butyric	1	1
Alpha-keto-gamma-hydroxy butyric	1	1
Succinic semialdehyde	1	1

found hydroxypyruvic acid as an artifact from aldol-condensation products of pyruvic acid (11). However, *Escherichia coli* has been shown to produce hydroxypyruvate from glyoxylate under anaerobic conditions (10). Succinic semialdehyde can arise to a small extent from alpha-ketoglutaric acid during catalytic hydrogenation (29), but the amounts found were often higher than would be expected on this basis.

*Free Amino Acids.* Table II indicates the average amounts of free amino acids in wheat grains and excised wheat embryos. Glutamic acid was the most prevalent free amino acid in the excised wheat germ

TABLE II  
THE FREE AMINO ACID CONTENT OF WHEAT GERM AND INTACT WHEAT GRAINS  
(Moisture-free basis)

AMINO ACID	GERM	WHEAT GRAIN
	μg/g	μg/g
Glutamic	1570	45
Aspartic	897	118
Alanine	762	28
Gamma-amino butyric	280	1-2
Glycine	220	
Arginine	500-700	50-75
Asparagine		
Serine		
Valine		
Leucine (s)		
Tryptophan	200-500	25-40
Lysine		
Threonine		
Cysteine		
Ethanolamine		
Glutamine	100-200	
Tyrosine		
Alpha-amino butyric acid	100	25
Proline		
Phenylalanine		
Piperidine-2-carboxylic acid		

and, in contrast to glyoxylic acid, glycine was present in only minor amounts. In any case, however, the amounts of free amino acids exceed by many times those of corresponding keto acids.

The situation seems to be a little different in the intact seed, where aspartic acid is the predominant free amino acid, followed by arginine and glutamic acid. Other amino acids occurring in significant amounts were alanine, asparagine, serine, valine, leucine (s), and tryptophan. If other free amino acids as indicated by Tables I and II are present, their concentration is too small to be detected by the methods used.

Rohrlich (26) found 2.6 mg. per g. of free gamma-aminobutyric acid in wheat germ extract. This amount is about ten times more than the present authors have found in dry germ, but agrees well with the amount present in heavily moistened germ after 1 hour. The failure of DeVay (3) to detect gamma-aminobutyric acid in sound wheat seeds by paper chromatography may have been due to the relatively small amount of this compound present, in addition to the fact that he used phenol-water and 2,4,6-collidine-2,4-lutidine-water as solvents. Whether the increase he noted in gamma-aminobutyric acid in moldy wheat is due to the metabolic reactions caused by mold enzymes or to a mold-induced increase in moisture content which activated glutamic acid decarboxylase in wheat grain is not clear.

*Effect of Wetting on Free Keto and Amino Acids.* As can be seen from Fig. 1, wetting the germ to 18% moisture at room temperature results in rapid decreases in the keto-acids glyoxylic, alpha-ketoglutaric, and oxaloacetic. The amounts of glyoxylic and alpha-ketoglutaric acids decrease to about half of the original concentration in as little as 3

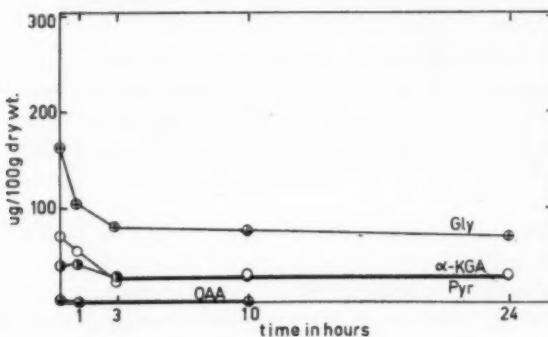


Fig. 1. Effect of wetting to 18% moisture on keto acids of excised wheat germ at room temperature ( $25^{\circ}$ - $27^{\circ}$ C.). Gly = glyoxylic acid; alpha-KGA = alpha-ketoglutaric acid; Pyr = pyruvic acid; OAA = oxaloacetic acid.

hours, following which the rate of disappearance slows down considerably. Oxaloacetic acid, which is always present initially at a relatively low concentration, could not be detected at all after 10 hours.

When the temperature was increased to 40°C., a condition which caused noticeable browning of the germ within only 24 hours, the rate of disappearance of alpha-ketoglutaric and oxaloacetic acids increased considerably. After 24 hours at 40°C. only 1/10 of the original amount of alpha-ketoglutarate could be found.

Changes in amino acid concentrations in wheat germ in 24 hours at room temperature when dampened to a moisture content of 18% are shown in Fig. 2. There is little if any effect on aspartic acid, but glutamic acid decreases at about the same rate as alpha-ketoglutaric

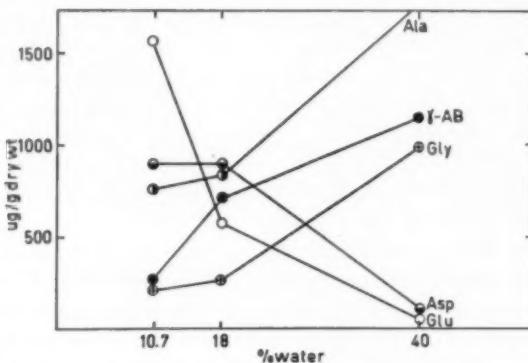


Fig. 2. Effect of moisture content on some free amino acids of excised wheat germ kept 24 hours at room temperature. Ala = alanine; gamma-AB = gamma-aminobutyric acid; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid.

acid. Gamma-aminobutyric acid increases moderately and alanine and glycine slightly. At 40% moisture content alanine, gamma-aminobutyric, and glycine increased sharply in the germ, whereas aspartic and glutamic acids decreased strongly. Further reference to these points will be made in the discussion of results obtained with intact grain.

The effect of increasing the moisture content to 39–40% upon the alpha-keto acids of embryos is illustrated in Fig. 3; that upon amino acids is illustrated in Fig. 4. Under these conditions, alpha-ketoglutaric acid almost disappears within 3 hours and is barely detectable after 27 hours at room temperature. The same is true of glutamic acid, the disappearance of which is accompanied by a spectacular immediate increase in the amount of gamma-aminobutyric acid (Fig. 4).

Figure 3 also shows that at 39–40% moisture and room temperature (25°–27°C.), oxaloacetic acid disappears completely within 3 hours after a barely noticeable maximum. Aspartic acid, on the other hand (Fig. 4), has a definite minimum at this point. The higher moisture has no further significant effect on glyoxylic acid, which under these conditions also decreases rapidly during the first 9 hours, followed, however, by a slight increase (Fig. 3). The initial decrease in glyoxylic acid could be partially due to the formation of hydroxypyruvate. However, no great accumulation of the latter could be detected. On the other hand, glycine, in Fig. 4, increases tremendously during the first 3 hours, probably owing to immediate activation of a transaminase.

It has been shown that the transamination between glyoxylate and

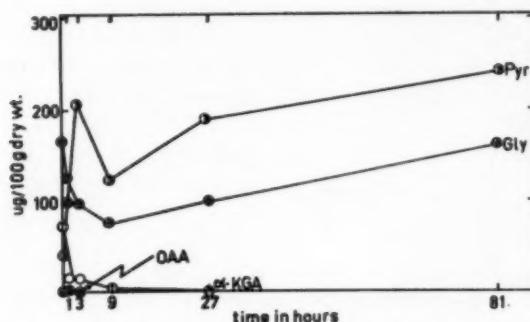


Fig. 3. Effect of wetting to 40% moisture content on keto acids of excised wheat germ at room temperature. See legend for Fig. 1 for key to abbreviations.

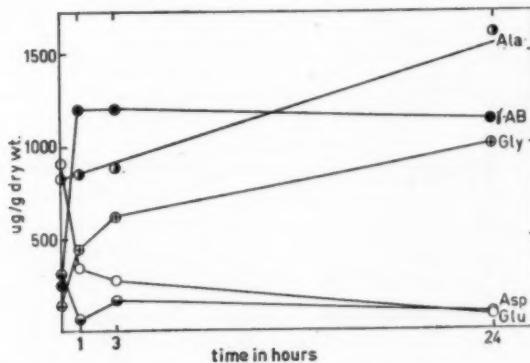


Fig. 4. Effect of wetting to 39% moisture content on some free amino acids of excised wheat germ. See legend for Fig. 2 for key to abbreviations.

glycine is in favor of glycine and can occur spontaneously in the presence of glutamate at room temperature (20). Recently, Kornberg and Krebs (9) presented evidence for the existence of a so-called glyoxylate cycle in *Pseudomonas* and this cycle has been shown to operate also in castor beans (8).

Alanine increases steadily in germ containing 40% moisture (Fig. 4). The semiquantitative analyses showed also an increase in the amounts of other amino acids released from proteins by hydrolysis, except for a drop in proline. This is in good agreement with the observation of Miettinen (15), who showed that in the pea plant in darkness glutamic acid, aspartic acid, and homoserine decrease, because photosynthesis and hence practically all of the primary synthetic

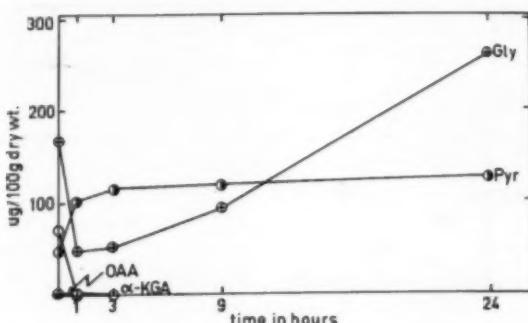


Fig. 5. Effect of wetting to 39% moisture content on keto acids of excised wheat germ under carbon dioxide atmosphere at room temperature. See legend for Fig. 1 for key to abbreviations.

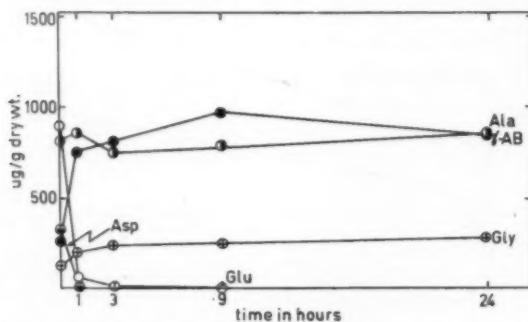


Fig. 6. Effect of wetting to 39% moisture content on some free amino acids of excised wheat germ kept under carbon dioxide atmosphere at room temperature. See legend for Fig. 2 for key to abbreviations.

processes are blocked. He found, however, that alanine, valine, and leucine (s) increase greatly, which could be caused only by enzymatic breakdown of protein.

*The Effect of Carbon Dioxide and Nitrogen Atmospheres.* The data of Figs. 5 and 6 show that if the germ is kept under carbon dioxide atmosphere during the wetting experiment the drop in alpha-ketoglutarate, oxaloacetate, and the corresponding amino acids is even more drastic than in air or under a nitrogen atmosphere. Exposing the germ to strictly anaerobic conditions is also accompanied by rapid evolution of hydrogen sulfide. A continuous increase in glyoxylate under carbon dioxide atmosphere, in contrast to final decrease under nitrogen, was also observed.

The data obtained from these experiments suggest an explanation for the immediate and rapid evolution of carbon dioxide characterized by elevated r.q. values immediately after wetting the germ, which has been recorded in previous studies (5,13,14). The main reaction most probably involved is the decarboxylation of glutamic acid. Alpha-

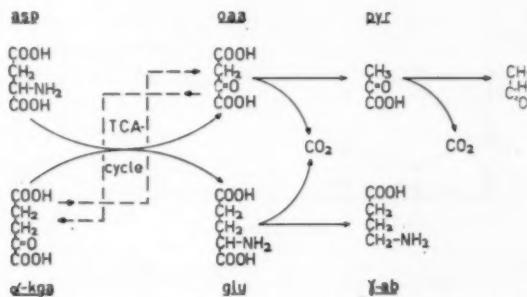


Fig. 7. Proposed scheme of changes occurring in free amino acids of excised germ due to wetting. TCA-cycle = tricarboxylic acid cycle; asp = aspartic acid; alpha-kga = alpha-ketoglutaric acid; oaa = oxaloacetic acid; glu = glutamic acid; pyr = pyruvic acid; gamma —ab = gamma-aminobutyric acid.

ketoglutaric acid would be removed from the scene by transamination with aspartic acid. The oxaloacetate formed may then decarboxylate to produce pyruvate, which in turn can either decarboxylate to acetaldehyde or form acetone via acetylcoenzyme A and acetoacetate. A possible scheme for these interactions is suggested in Fig. 7.

*Changes in Free Amino and Keto Acids During Germination.* To determine changes occurring in the free amino and keto acids during germination of wheat grain, seeds were surface-sterilized by soaking for 2 minutes in 0.1% solution of mercuric chloride, followed by rinsing

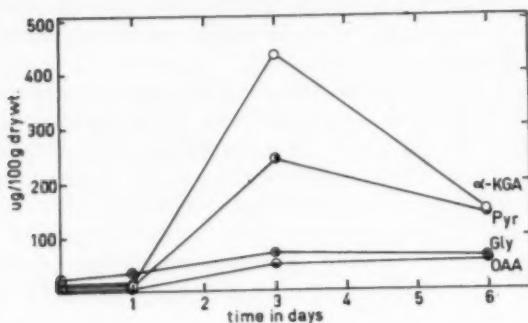


Fig. 8. Changes in keto acid concentrations in intact wheat grains during germination. See legend for Fig. 1 for key to abbreviations.

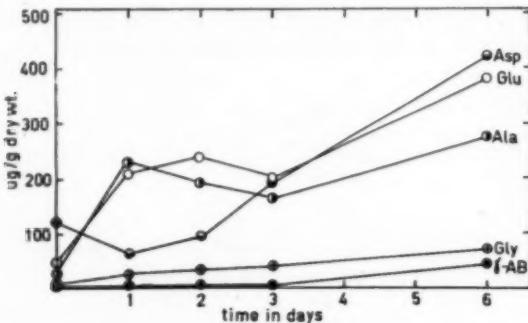


Fig. 9. Changes in the concentrations of some free amino acids in intact wheat grains during germination. See legend for Fig. 2 for key to abbreviations.

five times with tapwater. Seeds were then placed between filter paper sheets which were moistened every 12 hours. Germination took place under incandescent light, the green seedlings being about 3–4 cm. long after 4 days. Chlorophyll appeared after about 2–3 days. As shown in Fig. 8, the amount of all keto acids increased gradually at the beginning, under these conditions. Immediately after initiation of shoot growth there is a significant increase in alpha-ketoglutarate from less than 1  $\mu\text{g}$ . per 100 g. dry wt. originally to about 430  $\mu\text{g}$ . after 3 days (Fig. 8). As for amino acids, Fig. 9 shows that aspartic acid, glutamic acid, and alanine increased steadily, but that aspartate first passed through a minimum after 1 day, indicating the probable use of this compound for synthesis of other intermediates. Glutamic acid and alanine showed a slight minimum after 3 days, corresponding closely

to the maximum in alpha-ketoglutarate and pyruvate at this time. Gamma-aminobutyric acid and glycine increased only slowly until the 6th day, when the experiment was discontinued (Fig. 9). Semiquantitative analysis showed that serine, threonine, tryptophan (?), valine, leucine (s), tyrosine, lysine, and ethanolamine nearly doubled every 24 hours throughout the germination experiment. The amount of glutamine also increased significantly. It was originally present in minor amounts, but after only 24 hours it increased approximately tenfold. Asparagine passed through the same minimum as aspartic acid, but after 6 days it produced the strongest spot on the chromatogram. Arginine, which initially was one of the strongest spots, did not change noticeably during germination.

The general pattern of occurrence of free amino acids during germination of wheat seeds follows closely that which has been found in peas (32). The only exception is homoserine, which could not be detected in wheat. The situation is similar also in bacterial spores (6,23). Increase in many organic acids, including oxaloacetic acid, during germination of seeds has also been observed (21,22).

In the present studies, when only enough water was added to grains to produce a final content of 25%, all keto acids decreased after exhibiting a small initial maximum. The rate of decrease observed, which is very slow when compared to that shown by excised germ similarly treated, can, in the case of alpha-ketoglutarate and oxaloacetate, be greatly accelerated under a nitrogen atmosphere.

It is desired to stress the marked difference in pattern of change of keto and amino acids in excised germs and intact wheat grains due to wetting, and the fact that the degree of wetting and the nature of the atmosphere surrounding the material also profoundly affect the alterations observed.

Increases in the concentration of some amino acids when grain is wetted suggests that protein reserves are the source of these compounds. In this connection, McDonald and Milner (14) found that peptizable nitrogen values in grain decreased in direct relation to the increase in fluorescence and ultraviolet absorption of extracts of the grain, thus indicating a relationship to a browning reaction. Spores of *Bacillus mycoides*, which to a degree can be considered as similar in function to seeds, are known to be able to break down peptone, even though they otherwise are enzymatically inactive (34). The liberation of amino acids from proteins by protease activated by increased moisture content and the reaction of these compounds with reducing carbohydrates and glyoxylic acid may be a cause of brown pigments in wheat embryos. It has been postulated (14) that browning of cereal embryos is mainly

nonenzymatic, involving probably the formation of N-substituted 1-amino-1-deoxy-2-ketose through an Amadori rearrangement of the N-substituted glycosylamine formed by the interaction of amino compounds with reducing sugars. That such reactions can occur in a "dry" state has recently been shown (25). It seems logical that when storage molds are present they probably can both directly and indirectly accelerate the formation of brown pigments.

The breakdown of proteins with increased moisture must be related to a decrease in seed viability. According to Levinson (12), amino acids liberated from bacterial spore coats and proteins by a Mn<sup>++</sup>-activated lytic enzyme may be the primary stimulants for germination, and this is probably also true in higher plants. Under conditions of normal germination the liberated amino acids are transferred to the young seedling to be used for synthesis of important intermediates and new protein. If, however, there is not enough water to activate all the enzymes and provide the medium for translocation of the intermediates, the amino acids formed will react with available reducing carbohydrates to form, finally, the brown pigments characteristic of germ-damaged wheat.

The ease and rapidity of the loss in storage of important tricarboxylic acid cycle intermediates and related compounds, as well as the factor of protein breakdown, as indicated in this study, suggest that these processes are probably the major initial causes of deterioration and loss of viability in grain. Certainly the importance of glutamic acid for seed germination is well established (36). The present results seem to be useful in rationalizing the observation by Sorger-Domenigg *et al.* (27) that wheat dampened and then dried shows greater susceptibility to germ damage and mold growth in subsequent storage than does undampened wheat at the same moisture content.

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## STUDIES WITH RADIOACTIVE TRACERS II. The Presence of Unchanged Bromate in Bread<sup>1</sup>

C. C. LEE AND R. TKACHUK

### ABSTRACT

Experimental loaves of bread containing 100 g. flour were baked from a commercial, intermediate grade flour treated with 5, 10, 15, 20, 25, or 30 p.p.m. of Br<sup>82</sup>-labeled potassium bromate. Aqueous extracts of these loaves contain both bromide and bromate. Of the Br<sup>82</sup> activity in the extracts, the bromide accounted for 53-60%; the bromate contents ranged between 33 and 42%. Contrary to the earlier belief that all bromate used as improver would have decomposed after baking, these results indicate that some unchanged bromate is still present in the finished bread.

From qualitative tests or quantitative estimations by titration, it has been concluded that all the bromate is decomposed in bread made from flour containing bromate in concentrations used as improver. These views have been summarized by Hlynka (5). When bromate was initially present in excessively high levels, qualitative detection of residual bromate in bread was noted (4). With the more sensitive tracer technique, it was reported, in the first paper of this series (6), that radioactive silver bromate was precipitated, with the aid of carriers, from aqueous extracts of bread baked with Br<sup>82</sup>-labeled bromate as improver. However, because of the differences in self-absorption and sample geometry for samples prepared from solid silver bromate and samples prepared from drying aqueous solutions, accurate quantitative correlations of the silver bromate activity were not possible. The pres-

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ent paper reports data obtained by a procedure capable of giving quantitative estimates of both bromide and bromate in aqueous extracts of bread.

### Materials and Methods

An intermediate-grade,<sup>2</sup> untreated flour commercially milled from hard red spring wheat was used for all baking. It contained 14.0% protein and 0.46% ash (expressed on a 14% moisture basis). The choice of an intermediate rather than a patent flour was made because it is known that doughs prepared from lower-grade flours can decompose more bromate (3). If residual bromate were found in bread made from a flour of intermediate grade, one might reasonably assume that residual bromate would also be present in bread baked from patent flours.

The preparation of Br<sup>82</sup>-labeled potassium bromate, the baking of experimental loaves containing 100 g. flour initially treated with 5, 10, 15, 20, 25, or 30 p.p.m. of the labeled bromate, and the water extraction of the resulting bread were carried out as previously described (6).

For the determinations of bromide and bromate in the aqueous extracts, the bromide was first removed by exchange with bromine (1,2). This method has been shown to be applicable to the determination of bromide in aqueous extracts of bread and doughs (6). After the extract was free of bromide, the bromate was removed by precipitation as silver bromate. Measurements of the radioactivities in dried aliquots of the bread extract before and after bromide removal, and after removal of both bromide and bromate, gave data from which the contents of bromide and bromate in the extract could be calculated.

### Calculation of Data

To illustrate the method of calculation from the actual experimental data, loaf No. 1, which initially contained 5 p.p.m. (0.5 mg. in 100 g. flour) of labeled potassium bromate, is taken as an example.

*Total Bromate-Br<sup>82</sup> Activity Initially Added to Flour.* A solution was prepared by adding a known quantity of active bromate to an aqueous extract of nonradioactive bread. A "standard" sample was made by drying 1 ml. of this solution. This standard contained 1/800 of the 0.5 mg. of active bromate originally introduced in the baking formula. At an arbitrarily defined zero time, the activity of this standard sample was 1000 c.p.m. The total activity for the 5 p.p.m. bromate at zero time, therefore, equaled 800,000 c.p.m.

<sup>2</sup> Grade designations used by the mill from which the flour was obtained are patent, intermediate, and clear for the first 50%, the next 30%, and the last 12% of the total flour, respectively.

*Determination of Total Br<sup>82</sup> Activity in Bread Extract.* The total weight of loaf No. 1 was 141.8 g., of which 40.6 g. were extracted with 150 ml. of water. The extract was treated with 30 ml. of 1M zinc sulfate solution followed by neutralization with 46.3 ml. of 1M sodium hydroxide solution before clarification by centrifuging. Thus the total volume of the bread extract equaled 226.3 ml. Duplicate samples from drying 1-ml. aliquots of the bread extract were counted. The mean value of the observed activities was 821 c.p.m.

The standard sample was counted immediately before and after the counting of the samples prepared from the bread extract, and the mean activity of the standard was 990 c.p.m.

Corrected for radioactive decay to zero time, the activity of 1 ml. of the bread extract would be equal to  $821 \times 1000/990$  c.p.m.

Therefore, the total activity recoverable by water extraction from the entire loaf, corrected to zero time, would be  $821 \times 1000/990 \times 226.3 \times 141.8/40.6 = 655,000$  c.p.m.

The recovery of Br<sup>82</sup> activity in the bread extract was, therefore, equivalent to  $655,000/800,000 \times 100 = 82\%$  of the initial bromate concentration.

*Determination of Bromide-Br<sup>82</sup> Activity in Bread Extract.* To 5.0 ml. of the bread extract, 1.0 mg. of ordinary potassium bromate in 0.10 ml. of water was added as hold-back carrier and one drop of bromine (approximately 20 mg.) was introduced. The mixture was shaken thoroughly and then the bromine was removed by extraction with carbon tetrachloride. To aid the bromine removal, two drops of cyclohexene were introduced with the carbon tetrachloride. With the use of cyclohexene, a single extraction with 1 ml. of carbon tetrachloride followed by centrifuging was sufficient to remove all the bromine. Without the use of cyclohexene, the carbon tetrachloride extraction has to be repeated several times to remove all bromine coloration. Since any bromide present in the bread extract would exchange with bromine, the removal of the bromine effectively removed all the bromide. From the 5.1 ml. of bromide-free bread extract, duplicate 1-ml. aliquots were dried and counted. The mean value of the observed activities was 322 c.p.m.

The standard sample counted in conjunction with this determination showed a mean activity of 996 c.p.m.

Corrected for radioactive decay to zero time, the activity of 1 ml. of bread extract after bromide removal =  $322 \times 1000/996$  c.p.m.

For the entire loaf, the total activity remaining after bromide removal, corrected to zero time, would be  $322 \times 1000/996 \times 226.3 \times 141.8/40.6 \times 5.1/5.0 = 260,000$  c.p.m.

Since the total activity recovered in the bread extract at zero time = 655,000 c.p.m., the activity remaining in the bread extract after bromide removal =  $260000/655000 \times 100 = 40\%$ .

Therefore, the amount of bromide present in the bread extract =  $100 - 40 = 60\%$  of the total activity recovered in the extract.

*Determination of Bromate-Br<sup>82</sup> Activity in Bread Extract.* To effect the removal of bromate, 100 mg. of nonradioactive sodium bromate were dissolved in 1.5 ml. of the bromide-free bread extract. This was followed by the addition of 120 mg. of silver nitrate, with thorough shaking. The precipitated silver bromate was centrifuged out and 1 ml. of the supernatant dried and counted. This procedure was tested with a solution of a known amount of active bromate in an aqueous extract of nonradioactive bread. After the removal of the silver bromate precipitate, the activity in the supernatant amounted to less than 0.5% of the active bromate originally in the solution. For loaf No. 1, the activity of 1 ml. of the bread extract after removal of both bromide and bromate was 57 c.p.m. The mean value for the activity of the standard counted immediately before and after this determination was 952 c.p.m.

Corrected for radioactive decay to zero time, the residual activity in 1 ml. of the bread extract =  $57 \times 1000/952$  c.p.m.

For the entire loaf, the residual activity in the aqueous extract after removal of both bromide and bromate, corrected to zero time, would be  $57 \times 1000/952 \times 226.3 \times 141.8/40.6 \times 5.1/5.0 = 48,300$  c.p.m.

This is equivalent to  $48300/655000 \times 100 = 7\%$  of the total activity recovered in the extract.

Hence, the amount of bromate in the bread extract =  $100 - 60 - 7 = 33\%$  of the total activity recovered in the extract.

### Results and Discussion

Analytical data from 12 experimental loaves of bread baked with various levels of Br<sup>82</sup>-labeled bromate are given in Table I. Some of the duplicate loaves with the same initial bromate level showed fairly large variations in loaf volumes. This may possibly be due to difficulties in manipulating radioactive doughs while wearing rubber gloves. Nevertheless, increases in loaf volume resulting from the added labeled bromate were definitely observed. The total activity recoverable by water extraction of the bread and the bromide contents in these extracts were of the same order of magnitude as values the authors reported previously for bread baked from an experimentally milled straight-grade flour (6). For the twelve loaves analyzed, the bromide and bromate contents, respectively, ranged between 53 and 60% and

TABLE I  
ANALYTICAL DATA FROM BREAD BAKED FROM FLOUR  
TREATED WITH Br<sup>82</sup>-LABELED POTASSIUM BROMATE

LOAF No.	INITIAL BROMATE LEVEL (I.B.)	LOAF VOLUME <sup>a</sup>	TOTAL RECOVERY IN AQUEOUS EXTRACT	ACTIVITY IN AQUEOUS EXTRACT (RECOVERED ACTIVITY = 100%)		
				Bromide Content	After Removal of Bromide and Bromate	Bromate Content
p.p.m.	cc	% I.B.	%	%	%	%
1	5	845	82	60	7	33
2	5	875	82	55		
3	10	870	79	53	8	39
4	10	860	81	56		
5	15	875	84	57	8	35
6	15	890	85	56		
7	20	910	60 <sup>b</sup>	59	5	36
8	20	880	92	55	5	40
9	25	880	91	53	7	40
10	25	900	88	53	5	42
11	30	890	90	55	6	39
12	30	915	85	55	6	39

<sup>a</sup> Duplicate loaves baked without bromate showed volumes of 805 and 810 cc.

<sup>b</sup> Probably due to inefficient recovery resulting from materials adhering to the side of the Waring Blender out of intimate contact with the water during the extraction.

between 33 and 42% of the activity recovered in the extract. Agreements between duplicate samples with the same initial bromate level were quite good. In view of these results, it is reasonable to conclude that some unchanged bromate still remains in baked bread when potassium bromate is used as flour improver.

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## OBSERVATIONS ON FOOD PREFERENCES OF FIVE SPECIES OF STORED-PRODUCT INSECTS<sup>1</sup>

S. R. LOSCHIAVO<sup>2</sup>

### ABSTRACT

Food preferences of five species of stored-product insects among twelve milled cereal products and orientation responses of two species were observed in an apparatus that allowed insects to choose their food. Insect distribution was the criterion of measurement.

In an experiment with six cereal products, 30% of adults of the red flour beetle, *Tribolium castaneum* (Hbst.), were attracted to second-patent flour containing 5% wheat germ. In a three-food experiment, 46% of adults of the flat grain beetle, *Cryptolestes pusillus* (Schönh.) (often called *Laemophloeus pusillus*) were attracted to a formulated mixture consisting of 85% whole wheat flour, 10% wheat germ, and 5% brewer's yeast; 27% were attracted to whole wheat flour, and 24% to enriched second-patent flour. The attractive foods were relatively high in wheat-germ content. In a 12-food experiment with larvae of the meal worm, *Tenebrio molitor* L., 20% were attracted to each of fat-free bran and enriched second-patent flour, foods of low fat content; they were attracted to foods containing wheat germ in the absence of foods of lower fat content; of two foods differing only in fat content, 64% of the larvae preferred the one containing less fat. Larvae of the cadelle, *Tenebroides mauritanicus* (L.), had no apparent preference for any of twelve cereal foods.

Adults of *Tribolium confusum* Duv. and *T. castaneum* moved short distances toward preferred foods, probably in response to olfactory stimuli. Unattractive foods did not induce insect movement.

Mill-infesting insects become established in various cereal products that accumulate as "dead stock" in mill machinery. They are most abundant in nonmoving stock in elevator boots and screw conveyors. The degree of infestation depends on insect reproduction within that stock and on the number of insects that enter it. In mills, food-finding at appreciable distances is probably the result of random movement of insects and continuous movement of stocks, but at short distances insects may move toward foods in response to olfactory stimuli (3,15). Insects that accumulate in certain foods are the potential source of severe general infestations requiring expensive control measures.

In 1939, Shepard (9) found that wheat germ is particularly susceptible to infestations by cereal insects but that bran and other coarse materials are preferred, presumably because they are more easily penetrated. In 1941, Cotton (2) reported that clear and low-grade flours are attractive to several species of flour-mill insects.

Recently, Loschiavo (6) found that foods containing the largest amounts of wheat germ attracted the greatest number of flour beetles.

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<sup>2</sup> Associate Entomologist, Entomology Section.

Smallman and Loschiavo (10) confirmed the general observation by millers that millstocks high in wheat germ are highly susceptible to insect infestation. In 1953, Leclercq (5) found that adults of *Tenebrio molitor* L. preferred barley flour to either wheat flour or corn flour. In 1954 Magis (7) showed that *Tribolium castaneum* (Hbst.) preferred barley flour to corn flour and wheat flour to barley flour. Both Leclercq and Magis showed that food preferences were independent of humidity and thigmotaxis and attributed the response to a particular chemical sense.

The aim of this investigation was to determine the attractiveness of 14 cereal foods to five species of stored-product insects. Little information is available on insect food preferences, and since infestations in mills are most likely to develop in milling equipment carrying preferred foods, the results of this study should enable millers to carry out a more effective control program.

### Materials and Methods

The insects used were adults of the red flour beetle, *Tribolium castaneum*, and the flat grain beetle, *Cryptolestes pusillus* (Schönh.); and larvae of the yellow mealworm, *Tenebrio molitor*, and the cadelle, *Tenebroides mauritanicus* (L.). Table I shows a list of the foods used and their proximate analyses based on 14% moisture content. Whole-wheat flour and the mixture containing whole-wheat flour, wheat germ, and brewer's yeast were used only in the experiment with *C.*

TABLE I  
PROXIMATE ANALYSIS OF WHEAT PRODUCTS<sup>a</sup>

FOOD	PROTEIN (N × 5.7)	FAT	CRUDE FIBER	ASH	NITROGEN- FREE EXTRACT	
					%	%
Fat-free bran	13.5	0.2	12.1	6.2	54.0	
Enriched second-patent flour	11.2	0.9	0.4	0.4	73.1	
Second-patent flour plus 5% wheat germ	12.6	1.5	0.6	0.8	70.5	
Wheat middlings (feed)	16.3	5.3	4.8	3.4	56.2	
Coarse bran	12.4	3.1	12.4	5.7	52.4	
Fine bran	12.4	3.1	12.4	5.7	52.4	
First tailings stock	14.5	3.6	1.5	2.1	64.3	
First break stock	12.2	1.5	2.7	1.7	67.9	
Third break stock	13.4	2.2	4.9	2.7	62.8	
Coarse first middlings	10.8	0.5	0.4	0.4	73.9	
Fine first middlings	11.1	0.6	0.4	0.3	73.6	
First low-grade flour	14.9	2.2	0.5	1.4	67.0	
Mixture of 85% whole-wheat flour, 10% wheat germ, and 5% brewer's yeast						
Whole-wheat flour						

<sup>a</sup> Expressed on a 14% moisture basis.

*pusillus* and no analyses of these products were made. First and third break are stocks coming from the first break rolls and third break rolls respectively; coarse and fine first middlings are stocks going to the coarse and fine first middlings rolls respectively; first tailings is stock going to the first tailings roll; and first low-grade flour is stock coming from the first low-grade sifter. The other stocks used are finished mill products, namely, enriched second-patent flour, second-patent flour containing 5% wheat germ, coarse bran, fine bran, fat-free bran, a mixture of 85% whole-wheat flour, 10% wheat germ, and 5% brewer's yeast, whole-wheat flour, and wheat middlings. The latter product is the same as wheat gray middlings defined by the Association of American Feed Control Officials as consisting of fine particles of wheat bran, wheat germ, wheat flour, and the offal from the "tail of the mill." It contains less than 6% of crude fiber. Twelve of the foods were used with *Tenebrio molitor* and *Tenebroides mauritanicus*, six with *Tribolium castaneum*, and three with *C. pusillus*. The percentage of crude fat shown in Table I provides an estimate of germ content.

Food selection was determined by observation in the apparatus described by Loschiavo (6). Essentially it consisted of a cylindrical chamber 12 in. in diameter and 2 1/4 in. in height. In the center of the chamber was a raised stage 4 in. in diameter and 7/8 in. in height. The chamber was divisible into 12 sections or any multiple thereof by means of removable partitions extending radially from the stage to the rim of the chamber. Insects introduced upon the stage were free to move in and out of foods in the sections. As adults of *Tribolium confusum*, *T. castaneum*, and *C. pusillus* become unduly active for some time when disturbed, a special device was used to confine them on the stage during the period of undue activity. This device consisted of an open plastic ring 3/4 in. high and 3 3/4 in. in diameter. A plastic strip 1 in. wide was placed across the top of the ring along a diameter and glued at both points of contact with the ring. A 1/4-in. hole drilled at the center of the strip was fitted with a brass tube for introducing insects upon the stage. The insects were confined to the ring for 1 hour, which was sufficient time for them to return to normal activity. Then they were released and allowed to enter the foods in the sections surrounding the stage. After 48 hours the insects in each section were removed and counted. Because some of the pests are negatively phototactic, black covers were placed over the chambers.

The food selection tests were carried out at 32°C. and 70% relative humidity. Foods were held at these conditions for 2 weeks before each test to allow their moisture contents to come into equilibrium with that of the surrounding air. Because Willis and Roth (13,14)

showed that the physiological state of the insect is an important factor in eliciting hygrotactic responses, unstarved, undesiccated insects were used. Tests showed that altering the positions of the foods in the chambers had no effect on insect distribution. To avoid overcrowding, 50 larvae of each of *Tenebrio molitor* and *Tenebroides mauritanicus* were used in each chamber. In tests with *Tribolium castaneum* and *Cryptolestes pusillus*, 200 and 100 adults, respectively, were used in each chamber. Each figure in the data is the mean of 16 observations, four tests having been replicated four times. The data were analyzed by analysis of variance or the chi-square test, or both. Mortality that occurred in experimental insects was considered in the analyses.

Movement of adults of *Tribolium confusum* and *T. castaneum* from the central stage toward food was observed through a filter fitted over each chamber; as tests with differently colored filters showed that the movements of *T. confusum* and *T. castaneum* were unaffected by red or blue light, red and blue filters were used. Each of the two chambers was divided into two sections, one being filled with food and the other remaining empty. Bran and second-patent flour containing 5% wheat germ were tested separately. One hundred adults of each species, 6 weeks old and reared at 32°C. and 70% relative humidity, were introduced upon the stage of each chamber and confined there by a circular ring 4 in. in diameter. Their positions were observed at 48-hour intervals over a 12-day period to determine whether the insects elicit a directional response (taxis) to food.

### Results and Discussion

*Food Preferences of Tribolium castaneum.* Table II, column I, shows the distribution of 200 adults of *T. castaneum* in six wheat products. Second-patent flour containing 5% wheat germ attracted more insects than any of the others. Fine and coarse bran were significantly less attractive than any of the other four foods. Although bran was relatively unattractive, about four times as many insects were found in fine as in coarse bran. Except for enriched second-patent flour, foods high in wheat germ such as second-patent flour containing 5% wheat germ, wheat middlings, and first tailings contained significantly more beetles than did bran. Enriched second-patent flour contains at least three of the B-complex vitamins, of which thiamine hydrochloride has the most pronounced odor. Unpublished data at this laboratory showed that flour beetles are not attracted to enriched more than to nonenriched flour, but will move about and feed on pure thiamine hydrochloride separate from flour; they showed a similar response to alpha-tocopher-

TABLE II  
MEAN NUMBERS OF INSECTS ATTRACTED TO DIFFERENT FOODS

Food	SPECIES				
	Adults of <i>Tribolium</i> <i>castaneum</i> , 200 per test; 6 foods per test <sup>a</sup>	Adults of <i>Cryptolestes</i> <i>pusillus</i> , 100 per test; 3 foods per test <sup>b</sup>	Larvae of <i>Tenebrio molitor</i> 50 per test		
			III	IV	V
Fat-free bran			10	32	..
Enriched second-patent flour	49	24	10	17	..
Second-patent flour plus 5% wheat germ	61	..	3	..	22
Wheat middlings (feed)	46	..	3	..	9
Coarse bran	1	..	3	..	16
Fine bran	4	..	4	..	..
First tailings stock	39	..	1	..	..
First break stock	..	..	2	..	..
Third break stock	..	..	2	..	..
Coarse first middlings	..	..	3	..	..
Fine first middlings	..	..	2	..	..
First low-grade flour	..	..	2	..	..
Mixture of 85% whole-wheat flour, 10% wheat germ, and 5% brewer's yeast	..	46	..	..	..
Whole-wheat flour	..	27	..	..	..

<sup>a</sup> F = 213.00\*\*; d.f. = 5; 15.

<sup>b</sup> t = 4.20\*\*; d.f. = 5.

<sup>c</sup> F = 14.00\*\*; d.f. = 11; 33.

<sup>d</sup> t = 3.55\*\*; d.f. = 16.

<sup>e</sup> F = 9.80\*; d.f. = 2; 6.

ol. It would be interesting to explore the olfactory and feeding responses of flour beetles to vitamins.

The food preferences of *Tribolium castaneum* are similar to those of *T. confusum* (6). In 1945, Oozhuizen (8) showed that adults of *T. castaneum*, after a brief exploratory period in a food chamber, congregated in the finer milling products of maize and wheat. In 1947, Birch (1) showed that *T. castaneum* and *T. confusum*, placed in washed, medium-hard wheat, ate the embryo first and eventually reduced the kernel to a shell.

*Food Preferences of Cryptolestes pusillus.* Table II, column II, shows the relative attractiveness to *C. pusillus* of three wheat products. The mixture of 85% whole-wheat flour, 10% wheat germ, and 5% brewer's yeast was significantly more attractive than whole-wheat flour or enriched second-patent flour. Further work is necessary to determine whether B-complex vitamins in wheat germ and brewer's yeast and vitamin E in wheat germ (4) elicit olfactory responses in certain stored-product insects.

*Food Preferences of Larvae of Tenebrio molitor.* Table II, column

III, shows the distribution of 50 larvae of *T. molitor* in 12 wheat products. Fat-free bran and enriched second-patent flour, each containing 20% of the larvae, were significantly more attractive than the other ten foods. This experiment showed that foods high in wheat germ, namely, wheat middlings, flour containing 5% wheat germ, and first tailings, failed to attract significant numbers of larvae of *T. molitor*. That the two most attractive foods also contained low amounts of fat suggested that foods low in fat content are attractive to larvae of *T. molitor*.

This suggestion was tested in another experiment, which showed that fat-free bran was significantly more attractive than whole bran to larvae of *Tenebrio molitor* (Table II, column IV). The fat contents for the fat-free bran and the whole bran were 0.2 and 3.1% respectively (Table I). The two brans were alike in all other physical and chemical properties measured.

Table II, column V, shows the distribution of 50 larvae of *Tenebrio molitor* in three wheat products of different fat content. Second-patent flour plus 5% wheat germ, containing 44% of the larvae, was significantly more attractive than coarse bran or wheat middlings, containing 32 and 18% of the larvae respectively. The three foods in order of decreasing attractiveness contained 1.5, 3.1, and 5.3% fat, respectively (Table I). These results show again that larvae of *T. molitor* prefer foods of low fat content. When twelve wheat products were tested, these three were equally attractive to larvae of *T. molitor*. However, in the twelve-food experiment, 40% of the larvae preferred two of the foods of lowest fat content, namely, "fat-free" bran and second-patent flour, containing 0.2 and 0.9% fat, respectively (Table I); 53% were randomly distributed among the remaining ten foods; 7% died during the experiment. In the three-food experiment, 44% of the larvae again preferred the food of lowest fat content, namely, second-patent flour containing 5% wheat germ; 50% were distributed between bran and wheat middlings; 6% died during the experiment.

In the twelve-food experiment, first middlings containing less than 1% fat failed to attract mealworm larvae. The writer suggests that attractants were lacking in the endosperm portion of the wheat kernel from which first middlings is derived.

Though these experiments indicate that larvae of *Tenebrio molitor* were attracted to foods of low fat content, where the larvae had a choice between fat-free and whole bran, another interpretation is possible, namely, that they were repelled by whole bran. This interpretation is valid if food selection is influenced by repellent as well as attractant factors. Whole bran had a slightly rancid odor that was not

detectable in fat-free bran. The writer suggests that hydrolytic and oxidative breakdown of fat in bran produced odoriferous substances that repelled the larvae. Wardle (12) suggests that the term *host avoidance* may often be more accurate than *host selection*. In 1952, Loschiavo (6) found that with increasing rancidity bran became significantly less attractive to *Tribolium confusum*.

*Food Preferences of Tenebroides mauritanicus.* Larvae of *T. mauritanicus* had no apparent preference for any of the foods.

*Movement of Tribolium confusum and T. castaneum toward Food.* Figure 1 shows that in red or blue light more than 90% of the insects gathered at the edge of the central stage adjoining the section containing flour and wheat germ. The insects moved toward this food

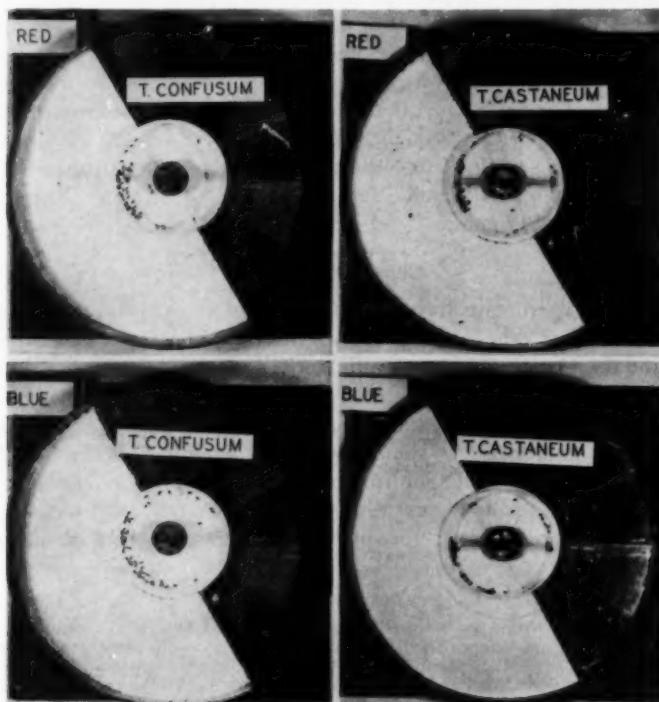


Fig. 1. Chambers uncovered to show positions of adults of *Tribolium confusum* and *T. castaneum* near flour containing 5% wheat germ, 1 hour after introduction upon the stage. The photographs were taken immediately after removal of the filters that covered the chambers.

immediately after their introduction upon the stage and remained near it until the experiment was terminated. When this food was replaced by bran the same insects became distributed at random over the whole stage. These observations indicate that *T. confusum* and *T. castaneum* perceive attractive foods at short distances, probably by olfaction. Fraenkel and Blewett (3) stated that unpublished experiments showed that *T. confusum* was attracted by the smell of flour in an olfactometer. Recently Willis and Roth (15) showed that the basiconic sensilla on the antennae of *T. castaneum* are olfactory receptors and suggested that these organs are responsible for olfactory responses in *T. confusum*. They state that the actual movement of *T. confusum* into food, from a position outside the food, may well be governed by some mechanism of olfactory orientation. Thorsteinson (11) concluded that an olfactory stimulus aids certain oligophagous insects in food-finding. Willis and Roth (13) were unable to demonstrate the attraction of *T. castaneum* by flour odor in an olfactometer, but they showed (14) that very dry flour, where hygrotaxis was not a factor, was slightly attractive. They explained their results as a possible olfactory response to flour. Since the foods in this experiment were kept at 70% relative humidity for 2 weeks before the tests, it was considered unlikely that moisture contents differed sufficiently to elicit stronger hygrotactic than olfactory responses.

### General Discussion

These experiments have shown that some cereal products are more attractive than others to certain mill-infesting insects. In mills, the size of the population in various mill stocks is determined by the number of insects attracted to the stocks as well as the favorableness of the stocks for survival, reproduction, and development (10). Insect distribution is determined by chance or choice, or both. The mechanics of mill operation (6), the continuous movement of stocks, and random movement of insects suggest distribution by chance (10). Under practical conditions, these factors may obscure the responses of insects to foods.

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## RELATIONSHIPS BETWEEN FAT ACIDITY VALUES AND TYPES OF DAMAGE IN GRAIN<sup>1</sup>

DORIS BAKER,<sup>2</sup> M. H. NEUSTADT,<sup>3</sup> AND LAWRENCE ZELENY<sup>3</sup>

### ABSTRACT

The fat acidity test was applied as a measure of different types of damage in corn, wheat, soybeans, and grain sorghums. Over 500 samples of grain, officially graded as to type and percentage of damage which they contained, were tested for fat acidity. In general, storage damage of the type caused by molds and heating shows high positive correlation with the fat acidity value, while field damage shows low correlation. Regression coefficients show the rate of increase which different types of damage produce in the fat acidity values. Positive correlations between fat acidity values and percentage of damage for some principal types of damage were: "sick" wheat, 0.847; heat damage (wheat) 0.651; heat damage (corn) 0.670; "rancid" damage (corn) 0.978; blue-eye mold (corn) 0.827.

Grain damage can be divided into two main categories: namely, field damage and storage damage. The latter is more important in terms of economic losses. The distinction between field and storage damage is emphasized by the causative agent. In a long series of papers on grain storage which have appeared in THIS JOURNAL, the latest being by Papavizas and Christensen (3), there has been striking evidence that molds under proper conditions of temperature and moisture are responsible for most storage damage in grain. Now Tuite and Christensen (4) have shown in wheat that very little mold infection takes place in the head of wheat in the field or in freshly threshed wheat, but infection does occur to a great extent in the country elevator and still more in the terminal elevator. Mold activity causes deterioration of the grain, and one of the best chemical indexes of this breakdown is the degree of hydrolysis of the fat or, as it is now widely expressed, the fat acidity (5).

An earlier paper (2) gave fat acidity levels characteristic of freshly harvested sound grain, the relation between fat acidity and certain types of damage in wheat and corn, and the application of the methods for determining fat acidity as a measure of soundness in grain. The present paper goes into greater detail concerning these relationships between fat acidity and different types of damage in corn, wheat, soybeans, and grain sorghums.

### Materials and Methods

Samples of grain were secured from several field offices of the Grain Division, AMS, with reports showing the type of damage and percent-

<sup>1</sup> Manuscript received July 28, 1958.

<sup>2,3</sup>Marketing Research Division and Grain Division, respectively, Agricultural Marketing Service, U.S. Department of Agriculture, Washington 25, D. C.

age of damaged kernels. In addition, some separations of particular types of damage were obtained as a basis for evaluating grain in which all kernels were damaged. Types of damage are designated by terms used by licensed grain inspectors and are defined in instructions issued by the U. S. Department of Agriculture.

It was difficult to obtain as many kinds of grain (showing evidence of a single type of damage) for this damaged-grain survey as were obtained for the sound-grain survey (2). Often lots of grain suffer more than one type of damage, and greater care is now being exercised in avoiding extensive damage in storage. However, more than 500 samples, principally corn and wheat, representing several types of damaged kernels were obtained. The fat acidity values were determined by the method described in *Official Methods of Analysis* of the Association of Official Agricultural Chemists (1).

### Results and Discussion

Some relationships between types of damage and fat acidity values appear in Table I. The correlation coefficients indicate the degree of the relationship between fat acidity and damage, while the corresponding coefficients of regression show the rate of change of the fat acidity values per unit change in damaged kernels. The regression coefficients are of particular interest in evaluating the sensitivity of the fat acidity test as an index of damage. This study shows clearly that the fat acidity value is generally a better measure of storage damage than it is of field damage. In corn, high correlations between fat acidity values and degree of damage are obtained for "rancid" damage, discoloration by heat, cob rot, and blue-eye mold. "Sick" wheat and "black germ"-damaged grain sorghums also show high correlations with fat acidity values. These types of damage are all classed as storage damage. A low correlation is obtained for weevil damage, which is a type of storage damage, because weevils are not consistent in selecting parts of the kernel to eat. They may eat part or all of the germ, thereby lowering both the fat content and the fat acidity. On the other hand, they may not disturb the germ at all, leaving the fat in the germ for attack by mold enzymes. These facts about weevil damage indicate that tests designed specifically to measure insect infestation would be more reliable for measuring weevil damage than is the fat acidity test.

Frost damage, a type of field damage, shows low correlation with fat acidity. Frosting of wheat apparently does not cause hydrolytic deterioration of the fat. Forty-seven of the 48 samples of frost-damaged wheat tested had fat acidity values below 20 regardless of the total amount of damage. A similar situation is observed in immature soy-

TABLE I  
RELATIONSHIPS BETWEEN DAMAGE AND FAT ACIDITY VALUES IN GRAIN

GRAIN AND TYPE OF DAMAGE	No. OF SAMPLES	DAMAGE RANGE			FAT ACIDITY VALUES			COEFFICIENT OF REGRESSION <sup>a</sup>	COEFFICIENT OF CORRELATION <sup>b</sup>	4-6% DAMAGE		100% DAMAGE	
		Min.	Max.	Mean	Min.	Max.	Mean			Min. F.A. Value	Max. F.A. Value	Min. F.A. Value	Max. F.A. Value
Corn		%	%	%	mg KOH/100 g dry grain								
Weevil	25	1.3	100	27.4	11	100	34	+0.267*	+0.447*	11	21	11	100
Rancid	30	3.5	100	13.4	20	138	43	+1.337**	+0.978**	20	43	..	..
By heat	55	1.0	100	19.6	16	173	45	+1.186**	+0.869**	16	49	76	173
Heat	31	2.0	100	45.5	24	275	81	+1.315**	+0.670**	27	29	49	275
Cob rot	38	2.2	100	30.6	19	272	98	+2.297**	+0.982**	19	38	224	272
Germ	27	3.0	95	15.9	24	99	50	+1.117**	+0.662**	29	37	..	..
Blue-eye mold	93	0.8	100	16.8	21	284	63	+1.510**	+0.827**	30	62	112	284
Ground	11	3.0	24	11.8	36	62	46	+0.261	+0.208	..	..	..	..
Wheat													
Heat	12	0.4	100	37.4	18	147	60	+0.628*	+0.651*	..	..	49	147
"Sick"	38	2.2	100	29.7	11	66	31	+0.339**	+0.847**	11	33	41	66
Sprout	34	2.0	100	18.8	7	45	21	+0.294**	+0.531**	10	21	16	45
Scab, blight	26	0.6	100	18.6	12	107	25	+0.391**	+0.650**	16	27	14	107
Frost	48	3.0	100	27.3	7	24	13	+0.050**	+0.453**	10	13	10	24
Soybeans													
Heat	11	2.6	100	58.6	19	182	65	+0.648*	+0.663*	..	..	56	182
Immature	15	1.7	12	4.6	12	26	17	-0.452	-0.299	13	24	..	..
Grainsorghums	31	0.5	92	11.2	20	171	56	+1.401**	+0.864**	37	51	25	..
Black germ													

<sup>a</sup> Regression of fat acidity values on percent damaged kernels (F.A.  $\times$  % damage).

<sup>b</sup> Fat acidity limit below which nearly all freshly harvested grain of unquestionable soundness is believed to fall (1).

beans. Immaturity, which is another type of field damage, shows an insignificant correlation with fat acidity, probably because the fat present has not been affected.

In the present system of grading grain for the factor "damaged kernels," the inspector examines the grain visually and handpicks damaged kernels. Percentage of damaged kernels is determined by weight. Licensed grain inspectors have specific instructions for distinguishing between sound and damaged kernels. However, there may be different degrees of damage in the damaged portion. On the other hand, the fat acidity test is an objective measure of the free fatty acids in grain, regardless of whether the damage can be detected visually, and is, in those cases cited, directly related to the extent of damage. For these reasons it is often difficult to correlate damage, as determined by the present system of inspection, with fat acidity values. Thus, it is possible to have two samples of grain showing the same percentage of damaged kernels, yet having widely differing fat acidity values because the extent of damage of each kernel was not ascertained by inspection. Such differences may be most pronounced in those samples designated as 100% damaged; see Table I where minimum and maximum fat acidity values are given for 100% damaged as compared to 4-6% damaged kernels. Furthermore, inspectors may differ among themselves as to the classification of damage in borderline cases. These facts may account for the low correlations obtained for heat damage, germ-damaged corn, and scab- and blight-damaged wheat.

In general, those types of damage caused by heating and molds show the highest correlation with the fat acidity value. The distinction drawn between damage "by heat" and "heat damage" is a matter of degree, "heat damage" being the greater degree. Some of the higher fat acidity values obtained for samples showing damage "by heat" indicate a greater degree of damage than slight discoloration by heat. The extent of damage caused by heating is probably more accurately measured by the fat acidity value than by visual examination.

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## QUANTITATIVE RELATION BETWEEN STRUCTURAL RELAXATION AND BROMATE IN DOUGH<sup>1</sup>

I. HLYNKA AND R. R. MATSUO

### ABSTRACT

The structural relaxation curve for a dough approximates a hyperbola which can be transformed to a straight line defined by a structural relaxation constant  $C$  (the intercept) and the asymptotic load  $L_A$  (the slope). It is now shown that the hyperbola's semiaxis,  $a$  (related to  $C$  as follows:  $a = \sqrt{2C}$ ), is a more useful parameter.

Since the semiaxis constant is a linear parameter, the value for a control dough may be subtracted from that for a bromated dough. The difference,  $\Delta a$ , yields a linear plot with initial bromate concentration for a given reaction time. Increasing the reaction time increased the slope of the line. Data are reported for doughs made from one flour, containing 0 to 30 p.p.m. bromate, and allowed reaction times of 5 minutes to 4 hours. Similar data have been obtained with other flours. Use of the semiaxis constant offers promising possibilities for additional studies.

One of the principal aims of dough rheology is to describe the properties and behavior of dough in meaningful and quantitative terms. A technique developed to this end is the method of structural relaxation (1-4) which is based on the observation that the properties of dough, that has been recently worked, change with time. Experimentally this change is followed by obtaining a series of extensograms for dough samples identically treated but given varying rest periods between shaping and stretching. If the extensogram load, at constant sample extension, is then plotted against rest period, a curve is obtained which describes the relaxation of dough.

It has been found that this structural relaxation curve is quite well approximated by a hyperbola (3). In order to make the relationship of the structural relaxation parameters to the hyperbola more readily understandable, a brief mathematical background is presented.

The equilateral hyperbola referred to its asymptotes as axis may be defined as a curve such that the product of the coordinates of any point on the curve is a constant, mathematically,

$$xy = C$$

If the curve is asymptotic to a line,  $y = b$ , the equation becomes:

$$x(y - b) = C$$

<sup>1</sup> Manuscript received July 28, 1958. Paper No. 172 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba, and No. 349 of the Associate Committee on Grain Research (Canada). Presented at the 43rd annual meeting, Cincinnati, 1958.

The relaxation curve is approximated (3) by an identical form of equation,

$$(L - L_A)t = C$$

where:  $L$  is the load at constant sample extension for rest period  $t$ ,  $L_A$  the asymptotic load, or the load approached at long rest periods, i.e. for a fully relaxed dough, and  $C$  has been termed the relaxation constant.

A more useful and convenient linear form is obtained by multiplying out and transposing terms:

$$Lt = L_A t + C$$

Thus, plotting  $Lt$  against  $t$  gives a straight line with slope  $L_A$  and inter-

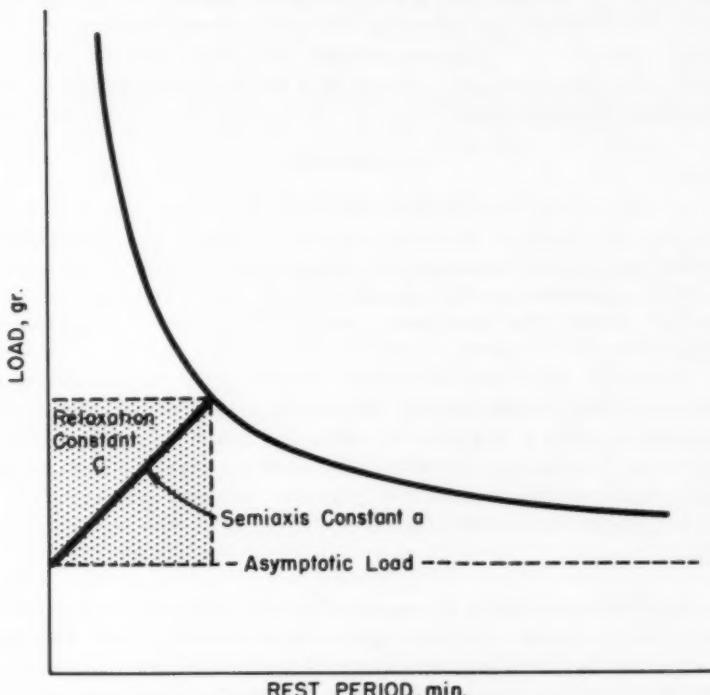


Fig. 1. A structural relaxation curve showing the relation of structural relaxation parameters. The asymptotic load  $L_A$  is the height the curve approaches at long rest periods; relaxation constant  $C$  is an area related to the curvature, and the semi-axis constant  $a$  is the distance from the origin to the vertex.

cept  $C$ . The slope and the intercept are calculated by the method of least squares.

Figure 1 summarizes graphically the essential features of the hyperbola (structural relaxation curve) and shows the relation of the rheological parameters. The constant  $L_A$  is the asymptote, representing the height the curve approaches at long rest periods, and the constant  $C$  is an area, a hyperbolic constant related to the curvature.

From consideration of first principles a new parameter, the hyperbola's semiaxis,  $a$ , has since been selected and has proved more useful. It represents the distance from the origin to the vertex of the hyperbola. Its relation to the relaxation constant  $C$  is given by the equation  $a = \sqrt{2C}$ .

If the value  $a_0$  obtained for a control dough is subtracted from the value  $a_x$  obtained for the same dough treated with bromate, then the difference,  $\Delta a$  represents the effect of the bromate on the dough. Since  $a$  is a linear parameter the subtraction is justified. This paper examines the relation between  $\Delta a$ , bromate concentration, and reaction time.

### Experimental

#### Materials and Methods

The flour used for this study was a straight-grade, improver-free unbleached sample, commercially milled from a blend of Canadian hard red spring wheat. The protein content of the flour was 13.1%, the ash, 0.50%. The absorption at 500 units on the farinograph was 62.8%.

Structural relaxation curves were obtained for doughs containing 0 to 30 p.p.m. bromate, in increments of 5 p.p.m., and allowed reaction times of 5 minutes, 1, 2, 3, and 4 hours. Methods used in obtaining the curves and in evaluating the relaxation constant  $C$  were those previously described (3). The semiaxis constant  $a$  was then calculated from the relation  $a = \sqrt{2C}$ .

### Results

Figure 2 summarizes the results obtained from structural relaxation curves. In the left-hand figure the increment of the semiaxis constant  $\Delta a$  is plotted against reaction time. For purposes of comparison the right-hand figure summarizes the same data in terms of the relaxation constant  $C$  used in previous studies (1-3). Although the two figures are superficially similar, the limitation in using  $C$  is that the data cannot be processed any further. Since  $C$  is a quadratic param-

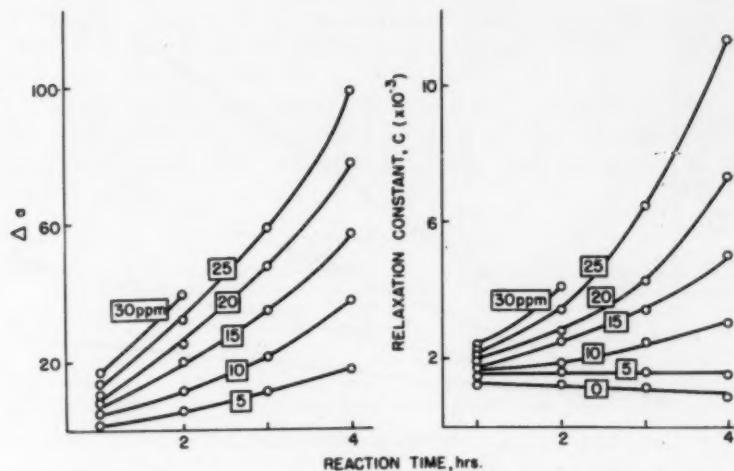


Fig. 2. Comparison of the two relaxation parameters for varying reaction times and fixed bromate concentration. On the left, the effect of reaction time on the subtracted value  $\Delta a$ , the semiaxis constant for the bromated less the semiaxis constant of the control dough; on the right, the effect of reaction time on the relaxation constant  $C$ .

eter, it cannot be directly subtracted to give simple and readily usable differences.

Values for the 5-minute reaction times were also obtained. They are not shown because of the small differences and relatively large uncertainties involved. Moreover, there appeared to be some residual effects on dough properties of the mixing process at the very short reaction times.

Figure 3, in which  $\Delta a$  is plotted against initial bromate concentration, presents striking experimental evidence of the advantage of the semiaxis constant. For a given reaction time,  $\Delta a$  is linearly proportional to the initial bromate concentration in the dough. This parameter, on the basis of this figure, is simply and straightforwardly related to bromate treatment and therefore is superior to the relaxation constant  $C$ .

The data for the 30 p.p.m. bromate at 3- and 4-hour reaction times are not shown. The results obtained at high bromate concentrations and at long reaction times appear to be outside the normal range of the structural relaxation method. The deviation from regularity becomes especially clear when tested by means of a linear plot such as that shown in Fig. 3.

This linearity between  $\Delta a$  and bromate concentration is not a

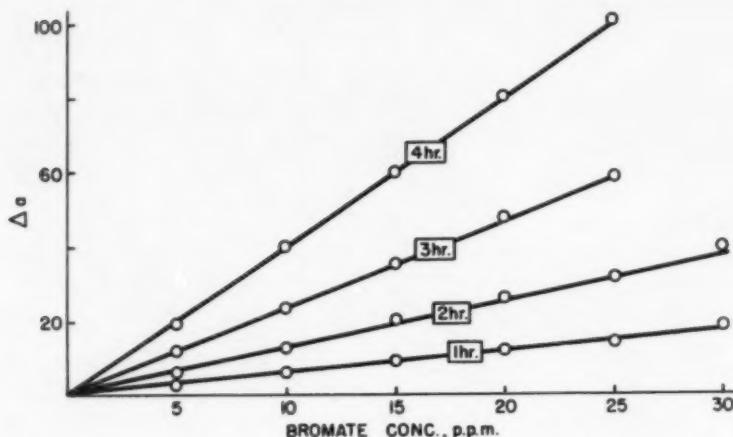


Fig. 3. Linear plot of initial bromate concentration against the increment of the semiaxis constant  $\Delta a$ ; i.e.,  $a_{\text{bromated}} - a_{\text{control}}$ , for fixed reaction times.

peculiarity of the flour studied. Structural relaxation data obtained for another flour tested by this method also gave a linear plot between  $\Delta a$  and bromate concentration. Analysis of other data from previous studies in this laboratory further confirmed this linearity. The linear relationship between the rheological parameter  $\Delta a$  and initial bromate concentration thus appears to be a general property of the dough system.

### Discussion

Although the relaxation constant  $C$  used in previous studies has been useful in describing the effect of bromate on the rheological properties of dough, it has not been possible to relate it simply to chemical treatment. With the semiaxis constant  $a$ , the linearity with the bromate concentration offers a simple means of recording the rheological effect of a given bromate treatment.

With the aid of the new parameter it now seems feasible to investigate additional fields. It seems possible, for example, that by selecting a specific bromate concentration and reaction time,  $\Delta a$  may be used to characterize the bromate responses of various flours in terms of a single figure.

The plot of  $a$  against bromate concentration in Fig. 3 may be considered as broadly analogous to a plot of loaf volume against bromate concentration in baking experiments. Both show the response

to bromate, the one in terms of physical dough properties and the other in terms of loaf volume. Further study is necessary to explore this interesting parallel.

Another challenging aspect of the results of this study is the potential usefulness of the semiaxis constant in kinetic studies of the reaction of dough with various reagents and again this aspect needs further study.

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## Cereal Chemistry

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**ERRATUM**

*Cereal Chemistry*, Vol. 36, No. 2  
March, 1959

Page 173, L. T. KISSELL:

In paragraph 3, beginning: "A negative linear relationship . . ." in line 3, the word *dioxide* should not appear. Deletion of this word will make the sentence correct.



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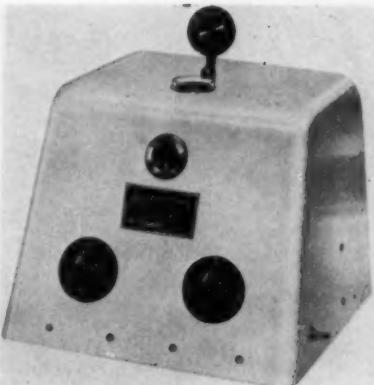
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